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IS THE HYPERSENSITIVENESS TO CHEMICAL AND PHYSICAL AGENTS ALLERGIC IN NATURE?

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DR. COHEN defined allergy as a state of altered reactivity of tissues to antigenic stimuli. This altered reactivity is mediated by antibodies which determine the specificity of this response. You have, no doubt, observed that among the substances which he has enumerated as capable of engendering the allergic state, he has mentioned drugs, which are not ordinarily considered to be antigenic; that is to say, they do not ordinarily stimulate production of antibodies upon parenteral introduction. This apparent discrepancy is the reason for the rhetorical question embodied in the title of my discussion. In brief, I propose to consider the question whether or not the immunogenic stimulus may be produced by an exposure to agents which are not antigenic by themselves.

On the basis of early observations it appeared clear that production of antibodies (and consequently the allergic state) can be engendered only by true antigens; that is, by complex colloidal nitrogenous substances of plant or animal origin (most characteristically represented by proteins containing aromatic amino acids). However, ever since the early description by Jadassohn of dermatitis medicamentosa in 1896, a rapidly increasing number of observations leave no doubt of the occurrence of a clinical syndrome elicited by an exposure to nonantigenic agents which cannot be distinguished from the ordinary allergic symptom complex, at least outwardly.

The first observation which threw light on the possibility that chemical substances may actually impress their specificity on immunologic processes was that of Obermeyer and Pick,[1] who showed that proteins, modified by introduction into the molecule of simple chemicals in vitro, may acquire new antigenic specificity determined by the identity of the chemical component. As a direct consequence of these findings, Wolff-Eisner[2] visualized the possibility that acquisition of sensitivity

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to nonantigenic excitants may be the result of remotely analogous interactions which may take place in vivo between the chemical excitants and the products of the exposed tissues. Because the actual mechanism of such in vivo conjugation is probably quite complicated and undoubtedly varies, depending on the nature of the particular agent involved, its nature remains obscure for the present. It is certain, however, that such in vivo synthesis does not involve any special mechanisms peculiar to the human species as claimed by many since it has been repeatedly reproduced in experimental animals.

It is true that sensitization of animals by direct exposure to chemical substances is not uniformly successful, yet Stumpke[3] has succeeded in sensitizing guinea pigs by intradermal injections of various nonantigenic plant extracts and Mu[4] has been able to sensitize rabbits to arsenicals by this route, although failing by other modes of exposure. Sulzberger[5] was particularly impressed by the fact that the outcome of such attempts is irregular and suggests that some undefined, possibly hereditary, peculiarities may be responsible for the individual differences in the capacity of animals to be sensitized. In a recent study, Jacobs, Kelley, and Sommers[6] showed that by selective crossbreeding they could obtain a stock of guinea pigs in which practically all animals could be sensitized with allyl isothiocyanate. However, these individual differences are not peculiar to animals, since in man, too, in spite of even prolonged occupational exposure or in cases of willful parenteral introduction of chemicals (as in chemotherapy), only a small proportion of those exposed become sensitized. Just what determines these individual differences is not known.

Since the experimental intracutaneous introduction of arsenicals sensitizes human beings with comparative ease (Natan and Munk[7]), it is not surprising that spontaneous sensitization by percutaneous exposure may be facilitated in individuals with pre-existing imperfections of the exposed surfaces of the skin (Burgess and Usher[8]).

Whatever the actual mechanism responsible for the sensitization with initially nonantigenic substances may be, it appears that, in the final analysis, in human beings as well as in animals the retention of chemicals in the tissues for a more or less prolonged period, as in the case of intracutaneous introduction, increases the likelihood of sensitization. Apparently local irritation with the following inflammatory tissue changes facilitates the formation of conjugates. Leideman[9] has found that tuberculin diluted in acacia sets up enough irritation in the tissues, especially if injection is made into tuberculous animals, to permit sensitization to gum acacia to take place.

That the status of the exposed tissue may play an important part in facilitating sensitization is suggested also by Jacobs,[10] who found that sensitization by iodinated sera is more effective in tuberculous guinea pigs by the method of Dienes than in normal animals. The fact that mere mixing of Lugol solution with serum in vitro results in an anti-

genic complex with the specificity of iodine suggests that in the presence of an inflammatory process the conjugation between the irritant and the tissue may not require a very complex process to occur, at least in the case of certain irritants.

All these facts seem to harmonize with Wolff-Eisner's hypothesis of in vivo synthesis of antigenic conjugates, but the validity of this hypothesis rests not merely on its plausibility. There are on record a few instances at least where the existence of these conjugates has actually been demonstrated. Thus in a case of allergic edema due to aspirin, Oriel[11] succeeded in isolating from the urine of the patient a proteose-aspirin complex which elicited a positive skin test in the patient, while either aspirin or the proteose alone failed to do so. Similarly, Rosenthal[12] has been able to demonstrate a serum-phenolphthalein antigenic complex in the blood of rabbits following the feeding or injection of the chemical. Moreover, synthesis in vivo of complex antigens is not limited to chemospecific conjugates; it has long been known that the Wassermann reaction depends on the detection of a lipotropic antibody produced as a result of stimulation of the tissues by an antigen synthesized during infection (Weil and Braun[13]). More recently analogous phenomena have been observed also in yellow fever (Hughes[14]) and in vaccinia infections (Smith[15]).

Further support of the validity of Wolff-Eisner's interpretation of the mechanism of sensitization to essentially nonantigenic substances is supplied by the recent investigations of Landsteiner, who succeeded in producing contact dermatitis to simple chemicals not only by direct intracutaneous introduction of the chemical, but also by systemic injection of the chemospecific conjugate. In the latter case dermatitis occurred upon introduction of the chemical alone into the skin (Landsteiner and Chase[16]). Incidentally, it should be noted that neither the animals sensitized intracutaneously by the chemical alone nor the animals rendered sensitive by a single intraperitoneal injection of the conjugate possessed demonstrable antibodies in the blood, thus recapitulating the conditions currently met in cases of so-called atopy in human beings.

These and other similar findings supply important evidence in favor of the view that the contact dermatitis type of response to nonantigenic substances *need not* be considered as a phenomenon apart from ordinary allergy.

It is clear that such a response is not inheritable but must arise as the result of previous exposure, recognized or unrecognized; it is not peculiar to human beings, but can be duplicated in experimental animals; its occurrence in the absence of demonstrable antibodies does not indicate that the phenomenon is independent of antibodies, but rather that the demonstration of antibodies is difficult, probably because they are present in a low concentration. Evidence for the latter statement is supplied by the experiment of Landsteiner previously

quoted, in which no circulating antibodies could be demonstrated by passive transfer from guinea pigs sensitized by means of the fully antigenic conjugate and exhibiting active sensitivity to contact with the chemical determinant of the conjugate. Because the sensitization in this case was secured by means of a complete antigen, no question could arise as to the fact that antibodies must have been produced, and if present in sufficient concentration, should have been able to transfer the sensitivity.

A direct proof of the participation of ordinary antibodies in the process of sensitization to simple chemicals is presented in a more recent experiment in which Landsteiner and Chase[17] have purposely intensified the process of sensitization by repeated intracutaneous introduction of a solution of citraconic anhydride in oil, whereby the antibody content in the blood of guinea pigs was sufficiently increased to permit passive transfer to other animals.

However, the failure to demonstrate the presence of circulating antibody is due not solely to its low concentration. Undoubtedly in many instances the failure to demonstrate passive transfer may be due to the fact that the allergen (or atopen) in question is an incomplete hapten and is unable both to stimulate the production of antibodies, and to produce a recordable reaction when brought in contact with antibodies produced in response to stimulation by an antigenic conjugate of which this incomplete hapten is the chemospecific determinant. For instance, in the experiment of Oriel referred to earlier, injections of the proteose-aspirin complex caused a positive skin test in the allergic patient in virtue of the "aspirin" specificity it possessed, yet aspirin itself (being an incomplete hapten) failed to give the reaction. Analogous findings have also been obtained by Remington,[18] who sensitized animals by repeated injections of aspirin conjugated with a protein, and the antisera of these animals precipitated in vitro the aspirin-protein complex but *not* the aspirin itself. However, the specific relation of the antibody to aspirin could be demonstrated by the fact that pure aspirin inhibited the precipitation of the aspirin-protein complex by the antiserum. Similarly, a simple mixture of Lugol solution with guinea pig serum acts as a complete antigen with iodine specificity and will sensitize guinea pigs, so that they can be shocked as the result of a subsequent reinjection of the iodinated serum (serum-Lugol complex), yet iodine itself not only fails to react directly, but also fails to inhibit the shock, while diiodotyrosine does inhibit the shock by serum-Lugol complex (Jacobs[10]). It is not surprising, therefore, that the passive transfer of serum from such an animal (sensitized by the serum-Lugol complex) fails to cause a response to iodine, irrespective of the amount of antibody transferred. On the other hand (provided the amount of antibody is sufficient to sensitize the skin), the injection of serum-Lugol complex, or of diiodotyrosine, might be expected to give a positive response.

While from the preceding discussion it follows that sensitivity to nonantigenic substances is determined by a mechanism entirely analogous to that responsible for establishment of sensitivity to complete antigens (provided that these substances undergo conjugation with the constituents of the exposed tissues and thus become complete antigens), it is not safe to assume that in all cases where the exposure to nonantigenic irritants is followed by symptoms resembling those encountered in allergic states one is necessarily dealing with true hypersensitiveness. That suggestive symptoms may after all be due to the primary toxic effect of the irritant and not to specific hypersensitiveness is illustrated by the following examples: When guinea pigs are given three or four intramuscular injections of benzene, xylol, or tuluol, the last injection may result in anaphylaxis-like symptoms and death of these animals. While the fact that repeated injections are necessary to elicit the symptoms suggests at first glance the establishment of immunologic sensitivity, Steiner[19] has found that actually these repeated injections have merely initiated an inflammatory process of sufficient intensity to permit a more rapid absorption of the chemicals. Thus, when the last injection was given, the material was absorbed quickly enough to cause a direct toxic effect resulting in a bronchospasm which simulated anaphylaxis. It is clear that in spite of an apparent alteration of response due to repeated exposure, in spite of the characteristic symptomatology, and in spite of the finding that symptoms and death were due to bronchospasm, these animals died, not as result of specific hypersensitiveness, but because of primary intoxication, as proved by the fact that in such animals the injection of epinephrine did not prevent the bronchospasm, since the latter was not due to a liberation of h. substance.

In general, then, while primary toxic effects may simulate certain symptoms encountered in the allergic state, repeated exposures to nonantigenic chemicals may result in establishment of a true allergic state. Because these chemicals in most instances are incomplete haptens, neither direct skin tests nor the Prausnitz-Küstner reaction can be elicited, so that recognition of the allergic nature of the symptoms is limited to empirical elimination, to subsequent willful test exposure, and to the patch test which permits slow synthesis of a complete hapten, which then reacts with sensitive tissue.

That certain individuals may exhibit unusual sensitivity to cold has been recorded for a long time. Munchmeyer[20] described urticaria in a soldier in the form of three large circular wheals upon the cheeks and chin, appearing after each exposure to a cold wind. Ungar[21] reports a case of a patient who developed extensive urticaria on exposure to cold air, followed in a few minutes by asthma. Upon return to a warm atmosphere, the shortness of breath gradually subsided and urticarial wheals vanished. Fraser[22] as well as Osler[23] has also discussed urticaria due to cold, and Ward[24] states that he has been seeing such cases since 1880.

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More recently, since the role of histamine-like products in allergy has been realized, many authors (Horton and Roth,[25] Roth and Horton,[26] Saylor and Wright,[27] and Horton and Brown[28]) have emphasized that the characteristic flush, acceleration of pulse, fall of blood pressure, increase of acid secretion in the stomach, and, in extreme cases, syncope, observed in cases of hypersensitiveness to cold, are quite similar to those observed when histamine is administered subcutaneouly.

That histamine-like products originate in the area injured by the exposure to cold has been suggested by the observation that if a tourniquet is placed above the exposed area, the symptoms remain localized, becoming generalized, however, upon release of the tourniquet and subsequent transport of these products to distant portions of the body (Bray[29] and Harris, Lewis, and Vaughan[30]).

The findings of Levine[31] as well as those of Saylor and Wright,[28] that washings obtained by injection and withdrawal of salt solution into the wheals produced by cold have a greater wheal-producing power than has plain saline, point in the same direction.

Because of this and other evidence, the mechanism of hypersensitiveness to cold has been widely considered to be allergic in nature, and in several instances the claim has been made that this hypersensitiveness can be passively transferred (Affolter,[32] Bernstein,[33] Lehner,[34] Covisa and Prieto,[35] Leibner,[36] Weissenbach and Brisset,[37] and Karady[38]). However, many others have failed in this and Harris, Lewis, and Vaughan[30] came to the conclusion that in uncomplicated cases of intolerance to cold, antibodies are not involved at all.

In some instances (usually as a complication of syphilitic infection), however, a truly immunologic response to cold is due to the presence of dermolytic or hemolytic (or sometimes both) antibodies which can unite with the specific cells only at low temperature and which require complement for the completion of the reaction which occurs when chilled tissue is brought to ordinary temperature of the body.

Another observation which has been cited in support of the immunologic background in cold sensitivity is the apparently successful desensitization by repeated exposure. However, since the ability of the skin to respond to histamine is known to be subject to "extinction" or "exhaustion" on repeated stimulation, the fact that such desensitization occurs merely strengthens the likelihood of the whealing itself being due to histamine but offers no additional evidence that allergy is the mechanism responsible for the liberation of histamine.

Thus, while the evidence for the allergic nature of hypersensitiveness to cold is far from being good, it does not preclude such an interpretation, provided it were possible to explain how the application of cold could result in the establishment of an allergic state (that is, if it were possible to recognize the antigen responsible for the sensitization) and for the response on subsequent exposure to cold.

Since exposure to nonantigenic chemical stimuli has been shown to result in the in vivo union of chemicals with the products of the in-

jured tissue, with the resulting formation of an antigenic product, it is not inconceivable that injury by physical means may so change the tissue as to impart to it a new specificity and thus render it auto-antigenic. In fact, it is known that even some of the normal tissues are auto-antigenic. For instance, one may produce antibodies in a rabbit by parenteral injection of its own lens protein. And as for antigenicity of abnormal tissue products, it suffices to recall that the antibody which permits the diagnosis of syphilis is the result of antigenic stimulation by the products of tissues modified by the spirochetal infection, and Bergmann[39] suggests that products of tissue which result from autolytic, degenerative, and other changes, and which in "Momentu nascendi" are "foreign" *(zustande-fremd)* may lead to autosensitization. For instance, urticaria, not unlike serum sickness, has been observed ten days after injury from a cricket ball (Whitefield;[40] also, Barbar[41]).

Also, Urbach and Steiner[42] claim to have shown that it is possible to produce antibodies and clinical sensitization to tissue products resulting from mechanical injury elicited by ingestion of pulverized glass. A similar possibility is suggested by Jacobs[43] to explain the fact that rabbits with tumors apparently produce antibodies against their own tissues. Antigenic stimulation by the autogenous tissue products probably explains also sensitization of women by their own milk in cases where lactation has been abnormal (Duke[44]).

That physical agents also may modify the immunologic specificity of antigens is well known. In fact, the use of coeto-antigens in forensic tests is based on the fact that denatured proteins possess an antigenic specificity different from that of the native proteins from which they have been derived. In line with these well-known facts, Karady[38] attempted to explain the phenomena of heat and cold sensitization as being due to the in vivo modification of the antigenic specificity of tissues, which rendered them auto-antigenic. This author claims to have been able to sensitize guinea pigs by immersing their extremities in cold water so that they exhibited symptoms of anaphylaxis upon a similar exposure three weeks later. Moreover, the author has also succeeded in accomplishing this antigenic modification of the guinea pig serum in vitro by exposing it for one and one-half minutes to cold (–5° C.) or to heat (56° C.) so that it could sensitize guinea pigs by parenteral route and elicit in them all the symptoms of anaphylaxis upon similar exposure repeated three weeks later. It is clear that evidence represented by Karady[38] should definitely prove the allergic nature of hypersensitiveness to cold (and to heat). Unfortunately, attempts to repeat these experiments in our own laboratory, as well as elsewhere (Richardson[45]), have been completely unsuccessful. Pending the confirmation of Karady's experiments and in view of the evidence presented above, one is forced to the view that while the symptoms exhibited on exposure to cold or heat closely resemble those

produced by histamine, the mechanism releasing the active substance seems to be other than the antigen-antibody reaction.

There is still another conceivable mechanism which may be responsible for local response to exposure to nonantigenic stimuli which resembles allergy. Auer[46] showed that in rabbits, sensitized to horse serum, production of local inflammatory area on the ear by gently rubbing its surface with xylol would result in necrosis of the area upon subsequent systemic introduction of horse serum, and Knepper[47] seems to have produced similar phenomena by exposing ears of the rabbit to heat (immersion in water at 40° C. for a half hour) or to cold (application of carbon dioxide snow to the thigh for a half hour) prior to intravenous injection of the antigen (pig serum). Klinge[48] and Klinge and Knepper[49] secured similar effects by producing a hyperemia of the ear of an allergic rabbit by denervation of one ear. However, in each of these experiments the authors failed to indicate what were the immediate changes observed; they merely found that rabbits killed three days after the test exposure to antigen exhibited focal eosinophilia, fibrinoid swelling of connective tissue with incipient necrosis, and inflammatory changes in the walls of blood vessels (Knepper).

It is not impossible that in some instances the individuals sensitive to true antigens may harbor free antigen in their blood. Production of an inflammatory focus in such individuals may result in sufficient accumulation of antigen in the focus to permit its union with antibodies to result in a local reaction, not unlike the Arthus phenomenon.

As to the mechanism responsible for release of histamine in heat and cold sensitivity, a plausible hypothesis has been offered by Grant, Pearson, and Comeau[50] which seems to harmonize with all the clinical observations. According to this hypothesis, the physical agents induce stimulation of efferent nerve fibers, releasing acetylcholine to the skin. This in turn releases histamine from the skin cells. In those individuals who exhibit an abnormally intensified response to a normal release of acetylcholine at the nerve endings, symptoms of histamine poisoning of varying intensity result. Thus the abnormality is presumably related to the peculiar functioning of the cutaneous tissue not unlike that observed in cases of dermographia where an excess of histamine is released in response to mechanical stimuli. The apparent desensitization observed on repetition of the stimulus may be either of very short duration and presumably due to refractoriness of the vessels or it may be more lasting (forty-eight hours) if it is due to a failure of the skin to respond to acetylcholine and the resulting failure to liberate histamine.

It seems, therefore, that the term cold or heat allergy is not justified by the known facts, and the term heat or cold intolerance should be substituted to avoid further confusion.

There are numerous reports that exposure to sunlight leads to urticaria, eczema, hives and prurigo, and occasionally even formation of

vesicles and bullae, referred to under the name of hydroya vacciniforme, also urticaria solaris. The most usual effect consists of erythema, edema, and itching, followed later by pigmentation limited to the exposed area. If the reaction is intense, general symptoms not unlike sunstroke may be elicited, and an intense local burn may result in necrosis. Thus, in general, in photosensitive individuals, reaction to visible light is identical with the reaction which normal individuals exhibit on excessive exposure to a short ultraviolet ray (Foerster and Schwartz[51]). As far back as 1862, Bazin[52] ascribed certain cutaneous reactions as being due to the effect of light, but the first attempt at explanation of this phenomenon goes back to 1909 when Ehrmann[53] suggested that hematoporphyrin, which was known to be present in such cases, may actually be the cause of the sensitivity to light.

Smith,[54] in referring to buckwheat poisoning in animals, stated that only white animals are affected and if they are kept in the dark or if artificially blackened, they escape the symptoms. He calls attention to the presence in buckwheat of a derivative of chlorophyll, phylloporphyrin, which resembles chemically hematoporphyrin and mesoporphyrin. Hausmann[55] injected hematoporphyrin hydrochloride into white mice and observed that they scratched themselves and their skin became edematous when they were exposed to the sun. In 1917 Meyer-Betz[56] gave himself an intravenous injection of 0.2 Gm. of hematoporphyrin and *immediately became* sensitive to light. While at first, blood and urine were positive for the porphyrin by spectroscopic examination, in a few days the drug seems to have been completely eliminated and both blood and urine were free. However, for several months exposure to light caused edema and itching, thus suggesting that hematoporphyrin must have been fixed by the tissues.

Ordinarily photosensitiveness may come into play either from endogenous sources (hematoporphyrin), or from exogenous sources (food). According to Low,[57] substances similar to phylloporphyrin, in addition to buckwheat, are also found in ordinary wheat and in oatmeal. Other instances of hematoporphyrinuria as the result of sulfonal, liver insufficiency due to syphilis or alcohol, congenital defects of liver (functional or organic), lead poisoning,[58] and quinine, acriflavine hydrochloride, and other fluorescent substances cause sensitivity to light.[59] Recently photosensitivity has been reported following sulfanilamide (Blum,[60] Goodman and Levy,[61] Schonberg,[62] and Laurens[63]).

If the results of experiments of Haussmann and of Betz reproduce the clinical picture of what goes under the name of hypersensitiveness to light, it is clear that it is not allergy, for it appeared immediately following chemical sensitization by injection. The mechanism of this phenomenon rests on the capacity of living cells to become sensitized to light so that longer visible rays, which are not ordinarily absorbed, become as effectively absorbed as are the short (less than 3,000 Å) ultraviolet rays (Edinow[64]). Presumably the injury consists in liber-

ation of histamine and an increase of permeability of superficial blood vessels with extravasation of plasma to the exposed site. This liberation of histamine may be due either to direct effect of light upon the tissue by transforming histidine to histamine through the agency of its quota of short ultraviolet rays (Holtz[65]) or indirectly through the intermediary of acetylcholine liberation from the nerves. In fact, Capps and Young[66] have actually found an increase in the histamine content of the blood and increased gastric acidity following exposure of the skin of a highly sensitive individual to the carbon arc lamp.

On the other hand, Abramson[67] doubts that wheal formation in photosensitive individuals is due to liberation of histamine because he failed to detect the presence of histamine in the lesions by (reverse) cataphoresis.

Beside the similarity in outward symptoms, the light sensitivity has been identified with allergy also because repeated short exposure temporarily increases tolerance to light. However, this tolerance is strictly limited to the previously exposed area, the rest of the skin remaining as sensitive as before.

Whether the physical explanation of photosensitivity is the *only* mechanism involved is difficult to say, especially since on the one hand there are cases of light sensitivity without porphyria and, on the other, there are cases where porphyrin is present in large quantities without sensitivity to light, and it has even been suggested that porphyrins may represent the result of skin injury by light rather than the cause of dermal sensitization (Mathews[68]). But in any event, it is very unlikely that any real allergy (production of antibodies) to light exists. Although several authors claimed to have succeeded in transferring light sensitivity passively, the lack of proper controls[69] minimizes the significance of such findings, especially in the light of findings of Callaway,[70] who has recently detected the presence of a photosensitizing substance in the blood serum of a patient with abnormal sensitivity to light, which could readily account for the apparent success of some passive transfers.

Although efforts to find abnormal light absorption bands in the serum of photosensitive individuals would represent a definite means of settling the problem of the nature of light sensitivity,[71] such a procedure seems to have limited only promise due to the fact that tissues retain photosensitizing substances for a considerable period beyond the time when they can be found in the blood or urine.[56]

REFERENCES

1. Obermeyer and Pick: Wien. klin. Wchnschr. **19**: 327, 1906.
2. Wolff-Eisner: Dermat. Centralbl. **10**: 164, 1907.
3. Steumpke: Klin. Wchnschr. **11**: 58, 1932.
4. Mu: Proc. Soc. Exper. Biol. & Med. **29**: 782, 1932.
5. Sulzberger: J. ALLERGY **6**: 39, 1934.
6. Jacobs, Kelley, and Sommers: Proc. Soc. Exper. Biol. & Med. **48**: 639, 1941.

7. Natan and Munk: Klin. Wchnschr. **7**: 1125, 1928.
8. Burgess and Usher: Canad. M. A. J. **23**: 45, 1930.
9. Leidman: J. Immunol. **38**: 237, 1940.
10. Jacobs: J. Immunol. **23**: 375, 1932.
11. Oriel: Proc. Roy. Soc. Med. **24**: 1171, 1931.
12. Rosenthal: J. Immunol. **34**: 251, 1938; Am. J. Path. **13**: 617, 1937.
13. Weil and Braun: Klin. Wchnschr. **44**: 1570, 1907.
14. Hughes: J. Immunol. **25**: 275, 1935.
15. Smith: Brit. J. Exper. Path. **13**: 434, 1932.
16. Landsteiner and Chase: J. Exper. Med. **73**: 431, 1941.
17. Landsteiner and Chase: Proc. Soc. Exper. Biol. & Med. **49**: 688, 1942.
18. Remington: Biochem. J. **34**: 838, 1940.
19. Steiner: J. Immunol. **27**: 525, 1934.
20. Munchmeyer: Berl. klin. Wchnschr., May 17, 1875.
21. Ungar: Berl. klin. Wchnschr., Nov. 28, 1881.
22. Fraser: Trans. Med.-Chir. Soc. Edinburgh **25**: 90, 1905-06.
23. Osler: Am. J. M. Sc. **127**: 16, 1905.
24. Ward: New York M. J. **81**: 742, 1905.
25. Horton and Roth: Proc. Staff Meet., Mayo Clin. **12**: 7, 1937.
26. Roth and Horton: Proc. Staff Meet., Mayo Clin. **12**: 129, 1937.
27. Saylor and Wright: Am. J. M. Sc. **192**: 388, 1936.
28. Horton and Brown: Proc. Staff Meet., Mayo Clin. **7**: 367, 1932.
29. Bray: J. ALLERGY **3**: 367, 1932.
30. Harris, Lewis, and Vaughan: Heart **14**: 305, 1929.
31. Levine: Arch. Int. Med. **56**: 498, 1935.
32. Affolter: Schweiz. med. Wchnschr. **63**: 881, 1933.
33. Bernstein: Dermat. Ztschr. **64**: 242, 1932.
34. Lehner: Klin. Wchnschr. **8**: 306, 1929.
35. Covisa and Prieto: Dermat. Wchnschr. **41**: 1188, 1930.
36. Leibner: Centralbl. f. Dermat. **34**: 406, 1930.
37. Weissenbach and Brisset: Ann. de Med. **32**: 333, 1932.
38. Karady: J. Immunol. **37**: 457, 1939.
39. Bergmann: Funktionelle Pathologie, Berlin, 1932, Julius Springer.
40. Whitefield: Lancet **2**: 122, 1921.
41. Barbar: Practitioner **209**: 1281, 1932.
42. Urbach and Steiner: Arch. f. Dermat. **153**: 772, 1927.
43. Jacobs: Proc. Soc. Exper. Biol. & Med. **47**: 88, 1941.
44. Duke: J. A. M. A. **98**: 1445, 1932.
45. Richardson: Proc. Soc. Exper. Biol. & Med. **45**: 787, 1940.
46. Auer: J. Exper. Med. **32**: 426, 1920.
47. Knepper: Virch. Arch. **296**: 364, 1935.
48. Klinge: Ergebn. path. Anat. **27**: 1, 1933.
49. Klinge and Knepper: Verh. deutsch. path. Ges. **28**: 181, 1935.
50. Grant, Pearson, and Comeau: Heart **2**: 253, 1936.
51. Foerster and Schwartz: Arch. Dermat. **39**: 55, 1939.
52. Bazin: J. A. M. A. **102**: 1103, 1934.
53. Ehrmann: Arch. f. Dermat. u. Syph. **97**: 75, 1909.
54. Smith: Arch. Int. Med. **3**: 350, 1909.
55. Hausmann: Biochem. Ztschr. **30**: 276, 1911.
56. Meyer-Betz: Deutsches Arch. f. klin. Med. **112**: 476, 1917.
57. Low: Anaphylaxis and Sensitization, Edinburgh, 1924, Green & Son.
58. Blum et al.: J. Clin. Investigation **14**: 435, 1934.
59. J. A. M. A. **102**: 1103, 1934.
60. Blum: J. A. M. A. **108**: 548, 1937.
61. Goodman and Levy: J. A. M. A. **109**: 1009, 1937.
62. Schonberg: J. A. M. A. **109**: 1035, 1937.
63. Laurens: J. A. M. A. **111**: 2389, 1938.
64. Edinow: Brit. J. Radiol. **3**: 112, 1930.
65. Holtz: Klin. Wchnschr. **12**: 1613, 1933.
66. Capps and Young: J. Clin. Investigation **19**: 778, 1940.
67. Abramson: Proc. Soc. Exper. Biol. & Med. **43**: 410, 1940.
68. Mathews: Arch. Path. **23**: 389, 1937.
69. Urbach: Wien, 1935, Maudrich, p. 137.
70. Callaway: Arch. f. Dermat. u. Syph. **41**: 889, 1940.
71. Arnold: Arch. f. Dermat. u. Syph. **43**: 607, 1941.

THE BASIC RELATIONSHIP OF ALLERGY AND IMMUNITY*

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IT IS my desire to present a definition of allergy with a working classification in which most of the immunologic phenomena which occur in animals and man can be placed and to discuss their interrelations. The ideas expressed here are not new or original; they have been published in part by others and in many instances have been either overlooked, discussed without appreciation of their importance, or simply forgotten.

I take as my major premise, to quote Rivers,[1] "The basic principles underlying serologic and immunologic phenomena in all fields of biology are identical. These principles, however, may evidence themselves in different manners in the various fields, and the techniques of studying the phenomena associated with them in different fields frequently vary because of the nature and mode of action of the infecting agents."

The term allergy was introduced by von Pirquet[2] to mean a specifically acquired altered reactivity. This broad definition has been so altered in usage that it is often difficult to understand another's usage of the term without a preliminary definition. This is true in no less a degree when we use such terms as atopy, desensitization, hyposensitization, anaphylaxis, and immunity. Some, for example, limit the term allergy to the clinical manifestations of hypersensitivity seen in man. Others use the term atopy for these cases. Still to others atopy means a hypersensitivity in animals or man which is determined by heredity.

Let us look at an air map of the "State of Allergy." It is only on such a map that we can see all the trails, highways, forests, hills and valleys, cities and towns in their proper perspective. Such a view may assist us in correlating our present ideas of the state and may furnish a more satisfactory use of our means of communication (Table I).

When an individual is infected by an organism or his cells are brought into contact with a nonliving, nontoxic antigen for the first time, no tissue reaction occurs, but a complex series of events is initiated. Living organisms without soluble toxins invade the tissues and set free certain chemical substances which stimulate the formation of antibodies. (In the case of nonliving, nontoxic antigens, it is the antigenic material itself which stimulates antibody formation.) It is not until specific antibodies are formed that specific tissue reactions occur and symptoms result. When antibodies are formed, the cells have a specifically acquired altered reaction capacity; allergy is present; the tissue response is allergic inflammation.

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TABLE I

Normal Cells plus Antigen	Incubation Period	Antibody Formation	Allergy			
				Plant and Animal Proteins, Drugs, Certain Bacterial Proteins	H-Substance	Reaction same for all antigens	Anaphylaxis; Serum disease; Clinical allergy
				Bacteria, viruses	Toxin	Reaction specific for antigen	Infectious disease
				Immunity	A quantitative allergic state in which the amount of H-substance or toxin is too small to produce changes in tissue. No signs or symptoms result.		

The correctness of this general statement can be tested by considering almost any infectious process. Let us take syphilis as our example.

Treponemas gain entrance to the body through a break in the skin or mucous membrane. Within a few hours they multiply and are to be found in the lymph channels and glands and are widely disseminated in the body. Despite the invasion by these organisms, their growth and dissemination, there is no clinical evidence of their presence for a number of days. Then the incubation period is ended; symptoms begin; a chancre develops. When the chancre begins, antibodies are present. This is evidenced by the fact that a positive serodiagnostic reaction for syphilis can be demonstrated by the most delicate tests in over 95 per cent of cases of primary syphilis. The chancre is the result of the tissue reaction to some toxic materials which are formed as the result of the antigen antibody union. This is an allergic reaction and is usually spoken of as a hypersensitive reaction. It is quite likely, however, as will be discussed later, that the tissues of a nonallergic animal would react in a similar way to these toxic products if they could be isolated and introduced to the nonallergic cells in the same concentrations. Under this conception, allergic reactions are due to toxic materials which are formed as a result of the interaction of antigen, antibody, and tissue cells.

The degree of reaction depends on the amount of toxic material formed, and this is determined by the quantitative relationships between antigen and antibody. Since, in most infections, the antigen is the source of the toxic material, the histology of the reaction is generally specific for the infecting organism and the degree of reaction dependent on the amount of organism present from which toxic material can be freed.

When an individual has a chancre, he has local allergic inflammation. If at this time other areas of his skin or mucous membranes are exposed to treponema, no reinfection occurs. In the area involved in the chancre, there are myriads of organisms from which large amounts of toxic materials can be freed by antibody action. In the reinoculated areas the antibodies destroy the few organisms which have entered the body before they can multiply. The amounts of toxic material formed are too small to injure the local cells; no reaction occurs; our patient has local tissue allergy and local tissue immunity at the same time.

From the foregoing example it can readily be seen that there is no fundamental difference between an allergic reaction and immunity. They are one and the same thing under different quantitative conditions.

Immunity is a subdivision of allergy in which antigen antibody reactions result in the release of too little toxic material to produce a reaction.

Cannon's studies of staphylococcus infections afford a beautiful experimental demonstration that the series of events just described for syphilis are characteristic of most infections.

A similar series of events and essentially the same fundamental process occurs in serum disease, anaphylaxis, and the clinical allergies. In all of these we see examples of the exposure of the body to an antigen, an incubation period during which antibodies are developed, and a reaction following antigen-antibody union. The characteristic difference between allergy as seen in infections and in the so-called experimental and clinical allergies lies in the nature of the toxic substance which induces the reaction. In the infections, as previously pointed out, the toxic material is formed in large part from the antigen. This determines the type of local and general reaction and characterizes the specific disease.

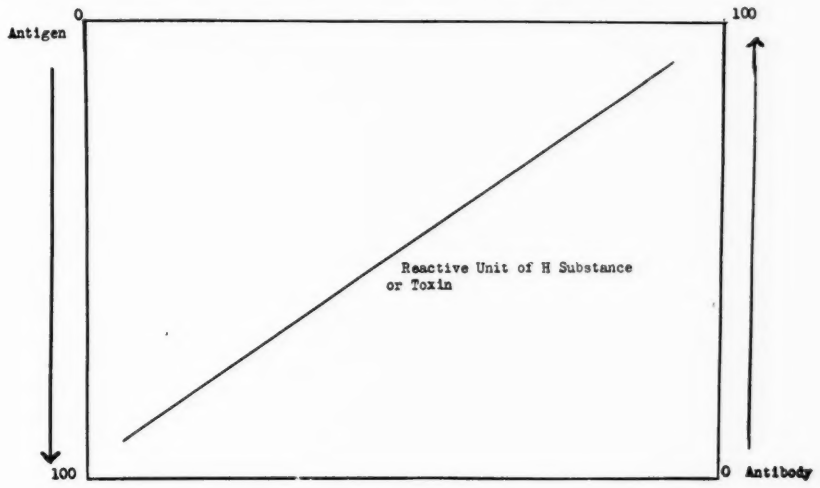

Fig. 1.

In the experimental and clinical allergies, the toxic substance released by the interaction of plant and animal foreign proteins, and drugs with their specific antibodies, is the same no matter how dissimilar the antigens may be. This accounts for the identity of the clinical symptoms and the pathology of the local lesions as has been shown particularly by Kline, Cohen, and Rudolph.[3]

A common substance formed under these circumstances could arise only from the tissues. The reaction might be called "body specific" in contradiction to the reactions in the infections which are "antigen specific."

There is considerable evidence that the toxic substances giving rise to body-specific types of reaction are identical with the H-substances described by Thomas Lewis. These behave in most respects like histamine, and in fact one can demonstrate no significant difference between histamine reactions, H-substance reactions, and those seen in the experimental and clinical allergies.

Reactions indistinguishable in all respects from allergic ones can be produced in the skin of nonallergic persons by cholinergic stimulation by a drug such as eserine. The amount of cholinergic stimulation de-

termines the quantity of H-substances formed and the severity of the reaction. This does not mean that histamine is necessarily identical with the H-substances or that the effect of the toxic substances released in the so-called allergic reactions is necessarily due in its entirety to histamine-like substances.

The cells are not hyperactive to the effects of H-substances or to histamine.

They are apparently hyperactive in the individual with a so-called clinical allergy only because they are exposed to a reactive amount of H-substance produced as a result of the antigen-antibody union. In passive transfer experiments normal skin reacts in an entirely comparable manner to similar amounts of H-substance.

Fig. 1 shows in a graphic way the proportionate realtionships of antibody and antigen which can produce a reactive unit of H-substance or toxin.

Antigen-antibody combinations which release less than the reactive unit produce no reaction. Our patient is immune. A slight quantitative change in the antigen antibody relationships operating under the same mechanisms produces symptoms. To illustrate this point, let us examine the skin test in ragweed hay fever. Let us suppose that our patient has had an intradermal test performed with an extract containing 0.0001 mg. of nitrogen per cubic centimeter and that no reaction occurred. We conclude that he is not sensitive. Therefore, he is locally immune to this dose of ragweed. If we repeat our test, however, using an extract containing 0.01 mg. of nitrogen per cubic centimeter, a definite reaction occurs. Our patient is now hypersensitive. When we treat a patient with a ragweed extract, he acquires a tolerance for increased amounts of this antigen. When the antigen is combined in this way with the new antibody resulting from the treatment, it cannot combine with the reagin to initiate the production of H-substances. Our patient is now immune to large amounts of antigen.

I have presented a discussion of the general mechanisms underlying the infectious diseases and the clinical and experimental allergies. I have attempted to show that there is no essential difference between the mechanisms. All immunologic phenomena are allergic. Quantitative relationships determine the existence of hypersensitivity, hyposensitivity, anergy, and immunity.

REFERENCES

1. Rivers, Thomas M.: Immunity in Virus Infections, Science **85**: 107, 1942.
2. Von Pirquet, Clemens: Allergie, München. med. Wchnschr. **53**: 1457, 1906.
3. Kline, B. S., Cohen, M. B., and Rudolph, J. A.: J. ALLERGY **3**: 351, 1932.

STUDIES OF TRYPTIC AND PEPTIC DIGESTION OF EXTRACTS OF GIANT RAGWEED POLLEN

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IT HAS long been recognized that a knowledge of the chemical nature of the active constituents of allergens is of great theoretical and practical importance. It is also recognized that a knowledge of the effect of enzymes on the active constituents would shed considerable light upon the nature of the allergenic substances. One of the earliest workers in this field was Kammann[1] who reported that the action of proteolytic ferments on the albumin of rye pollen resulted in a substance 100 times more active than the original crude albumin. Numerous investigations since his day have indicated that the active material in allergens is a protein. This view was widely accepted until, to quote the words of Newell,[2] "into this peaceful scene burst the announcement in 1923 by Grove and Coca[3, 4] that all of the nitrogen of a pollen extract can be eliminated by digestion and thorough dialysis without lessening the atopic activity," as measured by conjunctival and intracutaneous tests.

Since the work of Grove and Coca, a number of investigators have conducted digestion experiments on miscellaneous allergens. In 1924, Black[5] reported that he was able to repeat the work of Grove and Coca. He did not publish the details of his experiments except to state that the residue after digestion and dialysis reacted in the same dilution as formerly when tested by intracutaneous tests and by the minimum concentration necessary to produce sneezing when sprayed into the nose of a sensitive patient. In 1926, Caulfeild, Cohen, and Eadie[6] reported that tryptic digestion of the albumin-proteose fraction of short ragweed reduced its activity as measured by the Dale method. Tryptic digestion of the proteose fraction completely destroyed its activity. They considered that the persistence of the reaction in the case of the albumin-proteose fraction was due to insufficient treatment with trypsin. In 1927 Melli[7] reported experiments in which extracts of June grass (*Poa pratensis*), wall barley (*Hordeum murinum*), red top (*Agrostis alba*), and a species of nettle (*Parietaria officinalis*) were digested with pepsin, trypsin, erepsin, and a mixture of trypsin and enterokinase. He also tried papain in combination with hydrocyanic acid. The first three enzymes produced no change in the skin reacting capacity of the extracts as measured by scratch tests; digestion with trypsin plus entero-

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kinase resulted in a definite diminution of activity; extracts digested with papain became totally inactive. He submitted no evidence that digestion actually took place in the case of those enzymes which produced no loss of activity. In a series of experiments from 1928 to 1930, Farmer-Loeb[8] found that orchard grass *(Dactylis glomerata)* pollen extracts digested with pancreas extract and then dialyzed gave erratic results (although usually positive) by the Dale technique. However, extracts which gave positive tests by the uterine strip method gave negative skin reactions in sensitive patients after eight to ten days of additional dialysis.

In 1930 Bouillenne and Bouillenne[9, 10] reported that incubation with pancreatin for three days and subsequent dialysis produced a small but definite reduction of the activity of dwarf ragweed pollen extract as measured by scratch tests. However, there was a very marked reduction in skin reacting potency if the digestion was prolonged to fifty days. In 1934 Moore and Unger[11] digested short ragweed and timothy pollen extracts with pepsin and also with papain. Each of these enzymes brought about a reduction in the activity of the extracts as measured by scratch tests on sixteen patients. In 1935 Benjamins, van Dishoeck and German[12] reported that peptic as well as tryptic digestion diminishes the action of pollen extract as measured by the minimum dilution of the pollen extract necessary to give a slight but definite reaction by the intracutaneous method. In 1937 Bernstein and Kirsner[13] described experiments in which an extract of a mixture of equal parts of large and small ragweed pollen was incubated with gastric juice for four hours at 37° C. They concluded that the pollen extract so treated gave slightly less marked reactions by scratch tests than did the original. From their data we would estimate the change to be more than slight, possibly as much as 50 to 90 per cent. In 1941 Rockwell[14] arrived at similar conclusions by endermal tests from a study of ragweed pollen extracted by pepsin in hydrochloric acid. He considered, however, that the loss of activity was due to oxidation and to lessened solubility of the antigen in acid rather than to proteolysis. In 1940 Thiberge[15] reported experiments in which ragweed pollen extract was artificially digested with pepsin and hydrochloric acid. He also digested ragweed pollen extract with pancreatin. Dilutions 1:1000 of these digests were tested intracutaneously on twenty-nine sensitive patients. He stated that in twenty-three instances the reaction was either reduced or obliterated by gastric digestion but intensified by pancreatic digestion. We are unable to tell from the tables in his article how this latter conclusion was reached.

Thus, it will be seen that, for the most part, other experimenters have reported results at variance with the original work of Grove and Coca, but none of them has attempted to adhere closely to the method described by Grove and Coca. At least as late as 1936 Coca[16] indicated

that he had not modified his original view, and on one occasion he stated that the original work was so carefully done and so rigidly controlled that a repetition was unnecessary.

The object of our experiments was to repeat and extend the observations of Grove and Coca on the digestion of ragweed pollen extract with only such modifications as seemed indicated. Their method was essentially as follows: A dwarf ragweed pollen extract was incubated with a measured amount of proteolytic enzyme solution in a test tube at "room temperature" for twenty-four hours after which the digest was thoroughly dialyzed in a collodion sac "at ordinary icebox temperature." The extract remaining within the sac was then assayed for total nitrogen and its activity compared with that of the original solution by intracutaneous and conjunctival tests. The same amount of enzyme solution was then added to a portion of the solvent equal to the volume of pollen extract used and the mixture incubated and dialyzed in the same way as was the pollen extract. The nitrogen content of this control, after correcting for change in volume during dialysis, was found to be the same, within the limits of the experimental error, as that of the digested and dialyzed pollen extract. They concluded that all of the pollen protein had been digested and dialyzed away, leaving behind only those nitrogenous constituents added in the form of enzyme. They were able to detect no difference of activity in the pollen extract so treated and the original extract.

MATERIALS USED

1. Pollen Extract.—Giant ragweed pollen was collected by one of us (H. L. H.) with great care to prevent contamination by other pollens and also to eliminate insofar as possible dust and other debris. The well-dried pollen was extracted thoroughly with anhydrous ether, absolute alcohol, and anhydrous chloroform. In this latter liquid the pollen floated and could be skimmed off. A small amount of carbon particles and other debris settled out and thus was eliminated. After a final extraction with absolute ether, the pollen was dried and kept in a desiccator until used. The extracts were made by extracting 4 Gm. of pollen with 100 c.c. of the extracting fluid described by Grove and Coca. The nitrogen content of the resulting extract was 0.43 mg. per cubic centimeter. The formula of their extracting fluid was

KH_2PO_4	0.363 Gm.
$Na_2HPO_4 \cdot 12\ H_2O$	1.431 Gm.
NaCl	5.00 Gm.
Phenol	4.00 Gm.
H_2O	to 1000.00 c.c.

The pH of this fluid was theoretically and actually 7.0.

2. Enzyme Solution.—U.S.P. pancreatin was assayed by the Sörenson titration method using skim milk as a substrate, and also by the method of Helmer[17] in which the change in the refractive index of

casein digests was determined. The pancreatin was found to be well above the standards set by the pharmacopeia. The pancreatin solution was prepared by adding slowly, stirring, 10 Gm. of the powder to 50 c.c. of the solvent described above, and allowing it to stand thirty minutes. This was then filtered through paper into a graduate containing a 0.5 cm. layer of toluene. A portion of this solution was analyzed for nitrogen. This was done each time a fresh pancreatin solution was made, and the nitrogen content, when the method was closely adhered to, was quite constant. Concentrated pepsin which had been assayed on coagulated egg white by the McMeekin[18] method and found to be 1:76,000 was supplied by Dr. Milnor Freeland of the Presbyterian Hospital of Chicago. A solution was made from this powder of such strength that when 1 c.c. was added to 10 c.c. of the pollen extract, the pepsin was in the ratio of 1:100 with respect to pollen protein.

3. Dialyzing Membranes.—Preliminary experiments indicated that the following factors influence the permeability of celloidin membranes: (1) the ratio of alcohol to ether in the solvent; (2) the rate and amount of drying; (3) the amount of shrinkage; (4) the distance of the particular portion of the membrane from the mouth of the tube if prepared on the inside of a test tube; (5) the number of coats applied; (6) the nature of the solution in which the membranes are kept after they are made.

The solution used by us for making the membranes was an 8 per cent solution of celloidin in 20 parts of alcohol to 80 parts of ether by volume. When this had dissolved, 33 parts more of ether were added. The membranes were made by pouring the celloidin solution into test tubes and immediately pouring it out, clamping the test tube, mouth downward, at an angle of 60° for exactly sixty seconds, sliding it over a suction tube for sixty seconds, and then allowing it to stand inverted on filter paper on a smooth hard surface for twenty-four hours. The membranes were then placed in distilled water for twenty-four hours before using.

Membranes of two classes, calibrated by the graduated series of dyes proposed by Brown,[19] were used for the experiments: (1) "A" membranes, in which two layers of celloidin were applied, which withheld neutral Congo red and litmus for twenty-four hours, but readily permitted analine blue, neutral red, and safranine to penetrate. The lower end of these membranes was slightly stained by Congo red. (2) "B" membranes which were of single thickness and had shrunk away from the side of the tube while standing overnight. They withheld analine blue, neutral red, and safranine and were not stained by these dyes. It may be stated that in preliminary experiments membranes were prepared by different methods which permitted 20 per cent, 66 per cent, 78 per cent, 82 per cent, and even 96 per cent of the pollen nitrogen to escape after thorough dialysis. The "A" membranes permitted 78

per cent and the "B" membranes 20 per cent of the pollen nitrogen to dialyze away respectively.

4. Glassware.—All utensils were rinsed with concentrated nitric acid and then with distilled water to eliminate metals toxic to enzymes.

PROCEDURE

Several preliminary trials were discarded because we were not convinced that digestion was complete. In these trials, however, there was a marked loss of activity of the digested pollen extracts as compared with suitable controls.

According to present theories, the action of an enzyme on its substrate is an equilibrium reaction as follows:

$$\text{Substrate} \underset{\leftarrow}{\overset{\text{(enzyme)}}{\longrightarrow}} \text{split products.}$$

Hence in a closed system there would always be, theoretically at least, a residue of unchanged protein. Likewise the enzyme loses its activity rather rapidly at ordinary temperatures when in aqueous solution.

It was thought that by dialyzing away the split products as rapidly as formed and by adding more enzyme from time to time, the reaction could be carried to practical, if not absolute, completion. Pepsin was tried in addition to pancreatin in the hope that more clear-cut results might be obtained because of the fact that less nitrogenous material would need to be added in the form of enzyme solution. A trial in which portions of a 1:25 giant ragweed pollen extract were placed in an equal amount of strong buffer solution using each whole pH value from 1 to 13 and kept thus at room temperature for forty-eight hours and then in the refrigerator for eight weeks and finally neutralized and tested showed no marked loss of activity except below pH 2 and above pH 13. For this reason it was felt that the hydrogen-ion concentration necessary to secure peptic activity (pH 2) did not in itself cause any appreciable loss of activity. Peptic digestion is known to take place rapidly at pH 2, although we realize that this is not the optimum hydrogen-ion concentration for peptic activity.

Ten cubic centimeter portions of the 1:25 pollen extract were placed in five of the "A" membranes and five of the "B" membranes. These were dialyzed in small cylinders against an equal volume of solvent for three days. These dialysates were then assayed for activity and nitrogen content. There was no activity detectable by the scratch method in any of the dialysates. The nitrogen content of the dialysate was 0.118 mg. per cubic centimeter for the "A" membranes and 0.018 mg. per cubic centimeter for the "B" membranes with negligible variations among sacs of the same type. The sacs with their contents were then placed in large cylinders (Folin aeration jars) containing 70 c.c. of the solvent and dialysis continued at 3° C. with frequent stirring and daily changes

TABLE I

PROTEOLYTIC DIGESTION OF GIANT RAGWEED POLLEN EXTRACTS

SAC NO.	TYPE OF MEMBRANE	ENZYME	INCUBATION TIME (DAYS)	DIALYSIS TIME AFTER INCUBATION (DAYS)	WIDTH OF WHEALS IN MM. (SCRATCH METHOD)					% OF ORIGINAL STRENGTH	MG. N PER C.C.	
					JK	RP	RF	TG	AVE.		CONTROL	DIGEST
1	A	1 c.c. "20%" pancreatin	1	16	4 5	6 7	2 2	3 4	4.1	9	0.067	0.071
2	B	1 c.c. "20%" pancreatin	1	16	3 6	8 10	7 10	7 10	7.6	30	0.55	0.73
3	A	1 c.c. "20%" pancreatin	9	8	4 4	6 10	2 3	3 4	4.5	12	0.092	0.096
4	B	1 c.c. "20%" pancreatin	9	8	3 5	7 8	7 4	7 7	6.0	20	0.55	0.71
5	A	1, 1, ½ c.c.* "20%" pancreatin	3	14	6 4	8 8	3 3	5 5	5.2	15	0.139	0.139
6	B	1, 1, ½ c.c. "20%" pancreatin	3	14	3 3	14 14	6 6	6 5	7.0	27	1.21	----
7	A	9 daily additions* "20%" pancreatin	9	8	5 4	5 6	3 3	3 3	4.0	9	0.25	0.23
8	B	9 daily additions "20%" pancreatin	9	8	3 3	9 9	7 7	7 7	7.7	31	2.36	2.36
9	A	6 daily additions 0.01% pepsin*	9	8	1 1	11 12	4 5	3 4	5.2	15	0.018	0.072
10	B	6 daily additions 0.01% pepsin	9	8	1 2	8 11	5 6	10 10	6.6	24	0.055	0.45
		Original "4%" extract			10 10	17 17	12 17 12 20	7 9	13	100	0.43	
	A	Original "4%" extract after ten-day dialysis			11 14	8 9	9 13 9 14	11 13	11	65	0.092	
	B	Original "4%" extract after ten-day dialysis			15 20	7 7	11 15 10 17	10 10	12	85	0.36	

*1 c.c. first and second days: 0.5 c.c. each day thereafter.

of dialysate for seven days more. At this point 0.5 c.c. was removed from each sac for assay by skin tests and 0.5 c.c. for nitrogen content.

The ten membranes containing the 10 c.c. of dialyzed pollen extract were then divided into five groups with an "A" and a "B" membrane in each group (Table I). The following procedures were then carried out:

Group I (Sacs 1 and 2). One addition of 1 c.c. of a 1:5 pancreatin solution prepared as previously described was made to the dialyzed extracts, the mixture allowed to incubate and dialyze at room temperature (23° C.) for twenty-four hours, and then dialysis continued in the refrigerator (3° C.) with daily changes of dialysate for sixteen days.

Group II (Sacs 3 and 4). One addition of 1 c.c. of pancreatin solution was made with incubation and dialysis for ten days at room temperature, and then dialysis continued in the refrigerator for seven days. (Temperature control on Group IV.)

Group III (Sacs 5 and 6). One cubic centimeter of pancreatin solution was added on the first day, 1 c.c. more on the next day, and 0.5 c.c. more on the third day at room temperature; dialysis was continued for fourteen days in the refrigerator.

Group IV (Sacs 7 and 8). One addition of 1 c.c. pancreatin solution was made on the first day, 1 c.c. more on the next day, and 0.5 c.c. on each of seven succeeding days; dialysis was then continued one day more at room temperature and for seven days in the refrigerator.

Group V (Sacs 9 and 10). Ten cubic centimeters of pollen extract were dialyzed against a solution buffered at pH 2 for two days, and then 1 c.c. of pepsin solution of such strength that the pepsin was calculated to be in a ratio of 1:100 to the pollen protein was added. The next day 1 c.c. more was added and then 0.5 c.c. on each of four succeeding days. Dialysis was continued at room temperature for three days after which the dialysate was changed to a buffer solution of pH 7.0 and dialysis continued in the refrigerator for eight days more.

At the end of the dialyzing period, the final volume of solution in each of the ten sacs was measured and samples diluted with an equal volume of glycerin for assay for activity. The remainder was used for nitrogen analysis.

The entire procedure was then repeated with the solvent substituted for the pollen extract, using the same sacs, the same time, temperature, quantity of enzyme, etc. At the end of seventeen days the volume of the solution in each was measured and samples were taken for nitrogen analysis and for checking for sensitivity to the pancreatin or for possible pseudoreactions thereto. Some of the original pollen extract was taken from the refrigerator and kept at room temperature for ten days to be used for comparison in the assay of the digests in order to be sure that any loss of activity was not due to temperature change. As a further control, to determine how much activity may have been lost by dialysis alone, 10 c.c. portions of the original pollen extract were placed

in four of the same sacs used in the experiment (two "A" membranes and two "B" membranes), and dialyzed under exactly the same conditions and for the same length of time as those to which pancreatin was added. The loss of activity under these conditions is indicated in Table I.

Scratch tests with the dialysates obtained by dialyzing 10 c.c. portions of the pollen extract for three days against an equal volume of solvent without changing the dialysate during that time were entirely negative. Likewise, scratch tests with controls containing pancreatin but no pollen extract were negative, showing that activity remaining after prolonged digestion and dialysis is not due to sensitivity to, or pseudoreactions from, the pancreatin itself. Hence, we believe that any loss of activity in the digested and dialyzed pollen extract could be due to no other cause than the action of the enzymes thereon. It will be seen from Table I that there is a very marked difference in the size of the wheals obtained from the digested material and the size of the wheals obtained from the material remaining after dialysis alone. In the case of the "A" membranes, the nitrogen values of the digests and the controls, after correcting for changes in volume during dialysis, are, within the limits of the experimental method, identical. These results confirm those of Grove and Coca. The "B" membranes were sufficiently impervious to retain a little more of the activity in each.

Serial dilutions of the original extract, in which each succeeding dilution was one-half of the preceding, were tested by the scratch method, in duplicate, on the same patients at the same time that the digests were tested. The widths of the wheals in millimeters were then plotted as graphs so that the reactions to all of the treated samples could be located on the graphs and thus estimated in percentage of the original. The values are entered in the appropriate column in Table I. It is interesting to note that the loss of activity was 85 to 90 per cent in the "A" membranes and 69 to 80 per cent in the "B" membranes. The loss was about the same in those tubes incubated for ten days with nine additions of enzyme as in those incubated for one day with only a single addition of enzyme. This is contrary to the findings of Bouillenne and Bouillenne. That there is any loss is contrary to the conclusions of Grove and Coca. The less profound alteration of the protein brought about by peptic digestion also caused a definite loss of activity. However, the molecules of the split products were evidently too large to pass readily through the membranes since the nitrogen content of the digests was greater than that of the controls.

DISCUSSION

In evaluating digestion experiments on allergens of any kind, we feel that the following points are important:

1. It is not correct to assume that results obtained with one allergen are transferable to another. In this respect our own work might be

criticized as not being an exact repetition of the work of Grove and Coca since we used giant ragweed pollen whereas they used the pollen of the dwarf species. To be sure, numerous experiments have been reported tending to support the argument for the identity of the allergens of these two species. Others, however, would seem to indicate that this view is not quite correct. Our own experience on this subject might be of interest. In some work subsequent to the digestion experiments herein reported, we examined the records of some 400 ragweed-sensitive patients, each of whom had been tested in duplicate by the scratch method with four different samples of both dwarf and giant ragweed pollen. Twenty-five per cent of these patients gave uniformly larger reactions to the dwarf species than to the giant. In some of these the reactions to the dwarf ragweed were strongly positive and to the giant ragweed, negative. The remaining 75 per cent of the patients gave approximately equal reactions to each species. *In none was the reaction to the giant ragweed larger than to the dwarf.* This would seem to indicate either that the allergen or allergens are present in greater concentration in the dwarf species than in the giant or, what is more likely, that there are one or more allergens which are common to the two and one or more allergens present in the dwarf pollen which are not present in the giant. If the allergens present in these two closely related species differ, it is certainly hazardous to assume that experiments on any one species apply to pollens in general.

2. Because of the fact that the action of an enzyme on its substrate is an equilibrium reaction, some provision for removing the products of digestion while the enzyme is in contact with the protein should be made. This condition is not satisfied if incubation takes place in a test tube and the digest is then immediately transferred to a dialyzing sac in the refrigerator.

3. We believe that no refinement of the intracutaneous test will make it as satisfactory as are carefully executed scratch tests for quantitative assay of pollen or other allergenic extracts because of factors inherent in the two reactions. To illustrate this point, the reactions to intracutaneous and scratch tests, obtained in each of two typical patients selected at random from many others, are shown in Fig. 1. The sizes of the wheals in millimeters are plotted as abscissae and decreasing concentrations of the pollen extract (each succeeding concentration being one-half of the preceding) as ordinates. The greater slope of the curves obtained by plotting the scratch reactions is apparent at a glance. It is obvious that the intracutaneous test is unsatisfactory for detecting any but gross variations in concentration. As stated by Arbesman and Eagle,[20] "serial tenfold dilutions or even hundred-fold dilutions produce wheals which differ but slightly."

Because of the unreliability of the intracutaneous test as a quantitative method of assay, we believe that our results are actually in essen-

tial agreement with those of Grove and Coca and that we differ chiefly in the interpretation thereof. From their own data we find that the conjunctival reaction of two of the three patients tested was less to the digested and dialyzed ragweed pollen extract than to the undigested extract. In one of the three patients tested by the intracutaneous

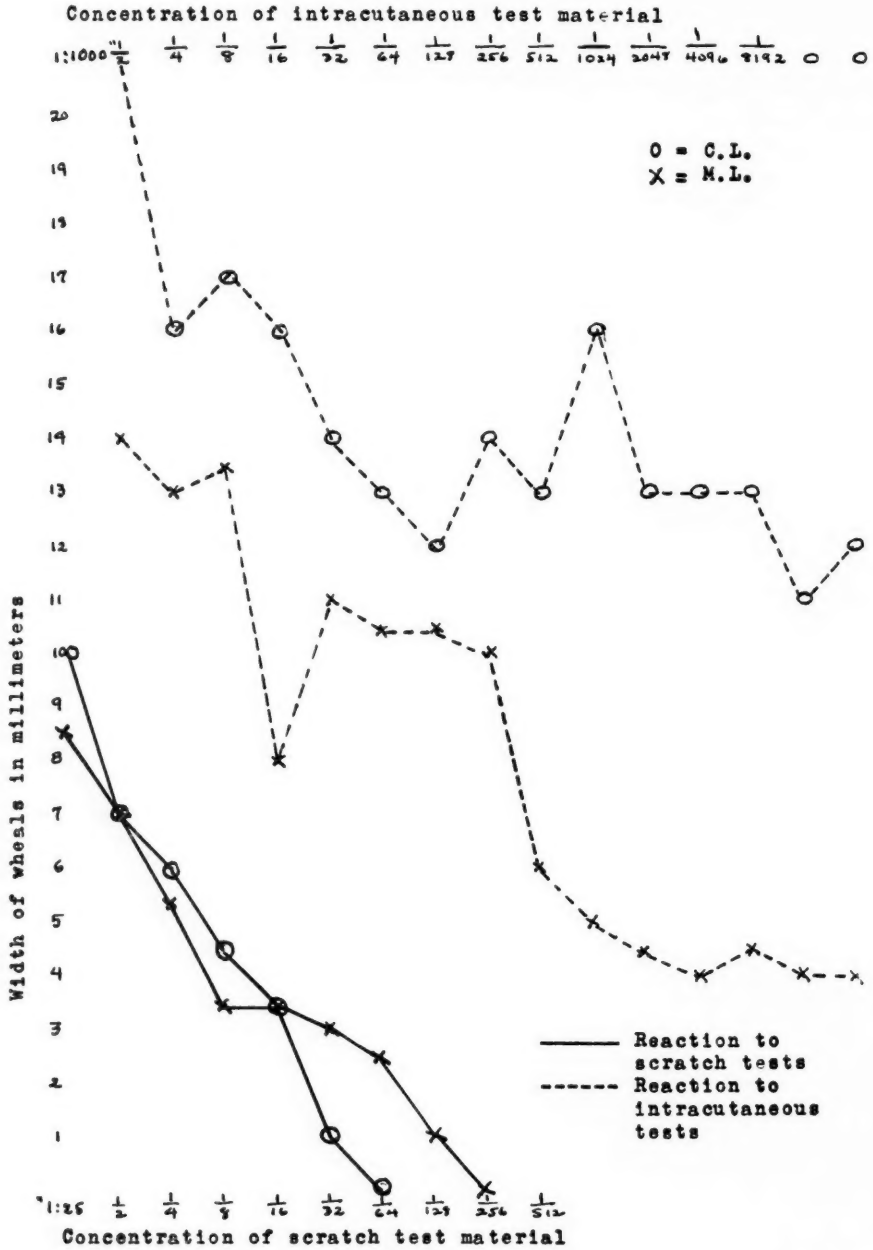

Fig. 1.—Comparison of scratch and intracutaneous reactions to serial dilutions of giant ragweed pollen extract in two typical cases.

method with the digested and undigested pollen, no difference between the two was detectable, but there was likewise no difference detectable between these two extracts and a 1:5 dilution of each. Obviously there could have been at least an 80 per cent loss of activity by digestion which would not be detectable by the intracutaneous method on this patient.

Finally, we must not overlook the fact that we, as well as Grove and Coca, find a small but definite amount of activity remaining after thorough digestion and prolonged dialysis. This small but constant activity may be due to active, nonprotein and nondialyzable prosthetic groups or possibly to molecules entirely independent of the protein. The nature of this active substance remaining after proteolysis might well be the subject of profitable investigation.

We believe that our results show that the major part of the activity of giant ragweed pollen is due to a digestible protein, to some substance inseparably associated with it, or to some substance active only in the presence of the protein.

SUMMARY

1. Giant ragweed pollen extract was subjected to digestion by freshly assayed pancreatin in calibrated dialyzing sacs. Digestion was also attempted with a highly active pepsin. In some cases as many as nine daily additions of enzyme were made to insure complete proteolysis. The digestion was followed by prolonged dialysis.

2. All digests showed a marked loss of activity which was not accounted for by dialysis alone. Nitrogen analysis indicated that digestion was complete in the case of tryptic digestion.

3. We cannot be certain that it was complete in the case of peptic digestion, because the membranes were not sufficiently permeable to permit all of the products of peptic activity to dialyze away.

4. There is a small but constant amount of activity in this pollen which is unaffected by proteolytic digestion.

REFERENCES

1. Kammann, O.: Weitere Studien über das Pollentoxin, Biochem. Ztschr. **46**: 151, 1912.
2. Newell, John M.: A Review of Chemical Studies on the Allergens in Pollens, J. ALLERGY **13**: 177, 1942.
3. Grove, E. F., and Coca, A. F.: The Nature of the Pollen Atopen, Proc. Soc. Exper. Biol. & Med. **21**: 48, 1923.
4. Grove, E. F., and Coca, A. F.: Studies in Specific Hypersensitiveness. XV. On the Nature of the Atopens of Pollens, House Dust, Horse Dander, and Green Pea, J. Immunol. **10**: 471, 1925.
5. Black, J. H.: Pollen Sensitization, J. Lab. & Clin. Med. **10**: 378, 1925.
6. Caulfeild, A. H. W., Cohen, C., and Eadie, G. S.: The Antigenic Properties of Pollen Fractions, J. Immunol. **12**: 153, 1926.
7. Melli, Guido: Sulla natura delle sostanze sensibilizzanti dei pollini, Riforma med. **43**: 511, 1927.
8. Farmer-Loeb, L.: Untersuchungen über die chemische Natur der Allergene. IV, Biochem. Ztschr. **220**: 432, 1930.

9. Bouillenne, M., and Bouillenne, R.: Recherches experimentales sur l'agent toxique du pollen d' ''Ambrosia div. sp.'' (Compositacées), Bull. Acad. roy. de méd. de Belgique **16:** 1052, 1930.
10. Bouillenne, M., and Bouillenne, R.: Ibid. **17:** 318, 1931.
11. Moore, M. B., and Unger, L.: Studies on Pollen and Pollen Extracts; Enzyme Digestion of Pollen Allergens, J. ALLERGY **5:** 338, 1934.
12. Benjamins, C. E., van Dishoeck, H. A. E., and German, J. L. M.: Studies on Active Substance of Grass Pollen; Activation of Small Molecular Weight Active Group Through Colloidal Substances, J. ALLERGY **6:** 335, 1935.
13. Bernstein, C., Jr., and Kirsner, J. B.: Oral Pollen Therapy; Experimental Considerations, J. ALLERGY **8:** 221, 1937.
14. Rockwell, G. E.: Effects of Enzymes on Ragweed-Pollen and Studies on the Iso-electric Point of Low-Ragweed Antigen, J. Immunol. **41:** 225, 1941.
15. Thiberge, N.: The Effect of Gastric Digestion on the Allergic Power of Pollen, New Orleans M. & S. J. **92:** 430, 1940.
16. Coca, A. F.: Discussion of Article on ''Chemical Standardization of Pollen Extracts,'' J. ALLERGY **8:** 87, 1936.
17. Helmer, O. M.: Doctor's Dissertation, University of Chicago, 1927.
18. McMeekin, T. L.: Doctor's Dissertation, University of Chicago, 1925.
19. Brown, W.: Preparation of Collodion Membranes, Biochem. J. **9:** 594, 1915; **11:** 40, 1917.
20. Arbesman, C. E., and Eagle, H.: The Assay of Ragweed Pollen Extracts, J. ALLERGY **10:** 521, 1931.

THE USE OF DRIED EXTRACTS IN THE PRACTICE OF ALLERGY

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THE problem of obtaining suitable extracts for the testing and treatment of allergic patients is one which confronts every practicing allergist. The ideal extract is one which is painless on injection and remains stable, potent, and sterile for a long time without the necessity of refrigerator storage. The very existence of numerous methods of preparation of extracts is in itself an indication of our ever-increasing efforts to obtain satisfactory allergens. Because of rapid loss of potency, it is necessary to prepare fresh extracts at frequent intervals. This is not only expensive and time-consuming, but also leads to constitutional reactions such as in the case of pollen extracts when one passes from a comparatively weak to a relatively strong extract. Glycerinized extracts delay and lessen loss of potency but are painful on injection. The glycerin in these extracts gives rise, on intradermal testing, to nonspecific reactions if used in a concentration greater than 5 per cent. Icebox storage is of value but only helps to delay rather than to prevent loss of potency. Furthermore, in the case of larger laboratories, it brings up the problem of sufficient storage space. This paper is concerned with a discussion of the usefulness of desiccation of extracts as a solution, even a partial one, to the problem as stated above.

LITERATURE

It has been known, of course, for a long time that proteins will not deteriorate if dried in vacuo. As early as 1896 Martin[1] suggested the possibility of preserving guinea pig complement in the dried state. Shackell in 1909[2] preserved complement for months after drying it in the frozen state. Cragie in 1931[3] dried complement from the frozen state in a vacuum desiccator. This method was applied to the desiccation and preservation of other biologicals by Elser and his associates.[4] Finally Flosdorf and Mudd[5] devised their "lyophile apparatus" which involves desiccation of materials such as biologicals through rapid freezing and dehydration under a high vacuum pressure. Ecker[6] more recently developed a fairly inexpensive and simple setup which may be made available in any average laboratory. This apparatus consists of a "high-vac" pump, a gallon sized aspirator bottle or suction flask which serves as a trap, and an ordinary vacuum desiccator. Granular barium oxide, which is used as desiccator, is relatively efficient as well as cheap, even

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though it cannot be regenerated. This method is efficient and inexpensive but is applicable to the drying of only small quantities of material. Tuft and associates[7] further reported on the use of the lyophile apparatus for preservation of reaginic sera.

It was natural that this general idea should be applied sooner or later to the preparation of allergic extracts. Thus Rappaport[8] reported that ragweed pollen extract rapidly dried by passing warm air over it at 40° C. and that it lost little of its activity when redissolved after eighteen months of storage. He arrived at this conclusion as a result of comparative direct skin tests on sensitive individuals. A few years later Cohen[9] indicated that he uses dust and pollen extracts that have been dried in vacuo. He states that "the dry material dissolves completely and has a potency unchanged when compared with fresh extracts."

TECHNIQUE

The procedure employed in the cryochem apparatus is similar in some respects to that of the lyophile apparatus. The material to be desiccated is dried in vacuo from the frozen state. Ten to twenty bottles or vials containing from 10 to 60 c.c. of extract each are connected to a manifold which is attached to a large desiccating chamber. The desiccant used is calcium sulfate. It can be regenerated by heat and therefore used over and over again. A high degree of vacuum (560 to 700 μ) is obtained by the use of a special vacuum pump. As the pressure is reduced, the material evaporates from the frozen state, and the water vapor thus given off is absorbed by the desiccant. The remaining material is left as a dry porous material. The bottles (infant feeding bottles are employed) or 10 c.c. viols are stoppered with rubber stoppers. The air may be withdrawn with a needle and syringe, and the tip of the rubber-capped bottle is dipped in paraffin. The original level of the fluid is marked on the bottle so that one may redissolve the material by the addition of a sufficient quantity of distilled water; a more concentrated extract may be obtained by adding a smaller quantity of water.

The method is simple in operation and relatively inexpensive. Two thousand cubic centimeters of extract may be dried by using 100 pounds of desiccant. The desiccant may be regenerated by heat and used over again for from twelve to sixteen times.

Small amounts may be redissolved at a time and suitable dilutions made up either for intradermal testing or for treatment purposes, such as in the case of dust or pollen extracts. In this way, fresh extracts are always available.

TESTS EMPLOYED TO DETERMINE ACTIVITY OF EXTRACTS

It is important to know, of course, whether extracts thus dried retain their potency when stored in the refrigerator and especially at room temperature. The comparative potency of these extracts can be determined by various methods. Of these, the direct skin test is the least

reliable. It is influenced by many factors, and for this reason it is not always an accurate index of the strength of the extract tested.

The total protein nitrogen likewise is not always a measure of the potency of an extract. However, total protein nitrogen determinations were made on saline ragweed extract at the time of preparation and six months later on a sample of extract stored at room temperature and in the icebox. As was to be expected, there was an appreciable nitrogen drop in the extract six months after preparation; the extract stored at room temperature showed a greater loss of nitrogen than the extract stored in the refrigerator. The cryochemed ragweed extract showed practically the same drop in nitrogen content. These data are presented without attaching too great significance to the nitrogen figure as an index of the activity of the extract in question.

The passive transfer method is, of course, reliable but cannot be used as a quantitative test. For this reason the neutralization test is considered more suitable for this purpose. It is based on the work of Coca and Grove,[10] of Cooke and his co-workers,[11, 12] and of Levine and Coca.[13] Briefly, this method of biologic assay of an extract is based on the fact that the strength of an extract such as pollen or dust can be determined by its neutralizing power on the serum of a sensitive patient. The technique of the test is as follows, assuming a given ragweed extract is to be tested:

A series of nine test tubes is set up. Into each test tube one adds 0.2 c.c. of the serum known to contain antiragweed reagins in great number. (This serum is obtained from a nontreated ragweed-sensitive patient and is tested for reagins by previous passive transfer tests.) The extract whose potency is to be determined is diluted serially, 1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280. To each tube containing 0.2 c.c. of serum is added 0.2 c.c. of this ragweed extract in concentrated form and in each of the dilutions stated. Tube 9, or the control tube, gets 0.2 c.c. of serum and 0.2 c.c. of normal saline. The tubes are shaken and allowed to stand at room temperature. One-tenth of a cubic centimeter of each mixture is injected intradermally into the skin of the back or the volar surface of the arm of an individual who is a known good receptor for passive transfer and who is not sensitive to ragweed. Two to five rows may be injected, using mixtures of the same serum and other ragweed extracts with which the original ragweed extract is to be compared. Twenty-four to forty-eight hours later each site is retested by injection of 0.05 c.c. of ragweed pollen extract in a dilution of 1:100 or 0.01 to 0.02 mg. of nitrogen. The presence or absence of a positive skin reaction is noted in terms of ± (doubtful), + (slight), ++ (moderate), +++ (moderate plus), ++++ (marked). It is obvious that the original amount of ragweed extract added to the reagin containing serum would neutralize these reagins. The amount or dilution of extract necessary to neutralize the reagins would, of course, depend on the strength of the extract. Two-tenths of a cubic centimeter of a 1:160 dilution may be sufficient in

TABLE I

NEUTRALIZATION TESTS SHOWING RELATIVE POTENCY OF SIX-MONTH-OLD POLLEN EXTRACTS

0.2 C.C. REAGINIC SERUM + 0.2 C.C. OF EXTRACT IN FOLLOWING DILUTIONS	MIXED RAGWEED				TIMOTHY				PLANTAIN			
			CRYOCHEMED				CRYOCHEMED				CRYOCHEMED	
	SALINE	GLYCERIN	ROOM TEMP.	REFRIG.	SALINE	GLYCERIN	ROOM TEMP.	REFRIG.	SALINE	GLYCERIN	ROOM TEMP.	REFRIG.
Concentrate	0	0	0	0	0	0	0	0	0	0	0	0
1:10	0	0	0	0	0	0	0	0	±	0	0	0
1:20	0	0	0	0	0	0	0	0	+	0	0	0
1:40	±	0	0	0	0	0	0	0	+	0	0	0
1:80	+	±	0	0	++	±	0	0	+	±	0	+
1:160	++	++	±	±	++	+	±	±	++	±	+	++
1:320	++	++	±	+	++	++	+	±	++	++	++	++
1:640	+++	++	++	++	+++	++	++	++	++	++	++	++
1:1280	+++	+++	+++	+++	+++	++	++	+++	++	+++	++	+++
0.2 C.C. SERUM + 0.2 C.C. N.S. (CONTROL)	+++	+++	+++	+++	+++	+++	++++	+++	+++	++++	++	+++
Potency of extract	1:40	1:80	1:160	1:160	1:80	1:80	1:160	1:160	1:10	1:80	1:160	1:80

the instance of one ragweed extract to neutralize 0.2 c.c. of the reagin containing serum. Testing a less potent ragweed extract, it would take, let us say, 0.2 c.c. of a 1:80 (or an extract twice as concentrated) to neutralize the *same* amount of the same serum. It is obvious if the serum is neutralized and all the reagins are destroyed that on retesting this site with a potent ragweed extract no reaction will occur. If, however, any reagins are left unneutralized, then these reagins will unite with the ragweed extract used later in retesting, and a reaction of variable degree will result. The appearance of such a reaction on retesting indicates, therefore, that the original dilution of extract used in that particular site was not sufficiently strong to bring about neutralization of the reagins. It may therefore be said that the minimum amount of pollen extract which will neutralize the fixed amount of reaginic serum may be taken as an index of the potency of that extract. Hence, if one tests, let us say, four different ragweed extracts, one may indicate their relative strength in terms of the dilution which was necesasry to neutralize the serum.

EXTRACTS TESTED

The neutralization test was used to determine the relative strength of several extracts. These included inhalants such as dust, feathers, and pollen extracts (mixed ragweed, timothy, and plantain); foods, such as milk and eggs, bananas, cashew nuts, and vegetables (cabbage, cauliflower, broccoli and Brussels sprouts). All extracts were prepared in June, 1940, according to Coca's method. The pollen extracts were divided into four batches: (1) saline, stored in refrigerator; (2) glycerinized, stored in refrigerator; (3) cryochemed, stored at room temperature; (4) cryochemed, stored in the refrigerator. The rest of the extracts were prepared by the same method on the same date. One-half of the extract was preserved in the refrigerator. The other half was cryochemed and stored at low room temperature.

Studies of comparative potency of the pollen extracts were carried out with samples which were six months (Table I) and twenty-two months old (Table II). Studies in comparative potency of the rest of the extracts were carried out with extracts which were twenty-two months old (Table III). In the case of cabbage extract, comparative tests were done with the fresh saline and cryochemed extract.

An examination of Table I reveals the comparative strength of the pollen extracts at the end of six months. The potency of each extract is expressed at the bottom of the table. The plain saline extract is weakest in each instance. The cryochemed extract stored at room temperature is somewhat stronger than the glycerinized extract. For the most part, cryochemed extracts stored in the refrigerator are somewhat stronger than any of the other extracts, although there is no appreciable particle difference between the two cryochemed extracts (i.e., when stored at room or refrigerator temperature).

TABLE II

NEUTRALIZATION TESTS SHOWING RELATIVE POTENCY OF TWENTY-TWO-MONTH-OLD POLLEN EXTRACT

0.2 C.C. REAGINIC SERUM + 0.2 C.C. OF EXTRACT IN FOLLOWING DILUTIONS	MIXED RAGWEED				TIMOTHY				PLANTAIN			
	SALINE	GLYCERIN	CRYOCHEMED		SALINE	GLYCERIN	CRYOCHEMED		SALINE	GLYCERIN	CRYOCHEMED	
			ROOM TEMP.	REFRIG.			ROOM TEMP.	REFRIG.			ROOM TEMP.	REFRIG.
Concentrate	0	0	0	0	0	0	0	0	0	0	0	0
1:10	0	0	0	0	0	0	0	0	0	0	0	0
1:20	+	0	0	0	+	0	0	0	±	0	0	0
1:40	+	+	0	0	++	+	0	0	±	0	0	0
1:80	±	+	0	0	++	±	0	0	+	+	0	0
1:160	++	++	±	±	++	++	+	±	++	++	+	±
1:320	++	+++	+++	++	+++	++	+++	++	++	+++	++	++
1:640	+++	+++	+++	++++	+++	++	+++	+++	++	+++	+++	+++
1:1280	+++	+++	++++	+++	++	+++	+++	+++	+++	+++	+++	++++
0.2 C.C. SERUM + 0.2 C.C. N.S. (CONTROL)	+++	++++	+++	++	+++	++++	+++	+++	+++	++++	+++	+++
Potency of extract	1:20	1:40	1:160	1:160	1:20	1:40	1:160	1:160	1:20	1:80	1:160	1:160

TABLE III

NEUTRALIZATION TESTS SHOWING RELATIVE POTENCY OF TWENTY-TWO-MONTH-OLD STANDARD AND CRYOCHEMED INHALANT AND FOOD EXTRACTS

REAGIN SERUM + EXTRACT TO BE TESTED IN DILUTIONS:	DUST		FEATHERS		MILK		EGGS		BANANAS		CASHEW NUTS	
	STANDARD	ROOM TEMP. CRY-OCHEMED	STANDARD	ROOM TEMP. CRY-OCHEMED	STANDARD	ROOM TEMP. CRY-OCHEMED	STANDARD	ROOM TEMP. CRY-OCHEMED	STANDARD	ROOM TEMP. CRY-OCHEMED	STANDARD	ROOM TEMP. CRY-OCHEMED
Concentrate	0	0	0	0	0	0	0	0	0	0	0	0
1:10	0	0	0	0	0	0	±	0	0	0	0	0
1:20	0	0	+	0	±	0	+	0	0	0	0	0
1:40	0	0	+	0	++	0	+	0	0	0	++	0
1:80	0	0	++	+	++	±	++	±	±	0	++	±
1:160	+	0	++	++	++	+	++	+	+	0	++	+
1:320	++	++	++	++	+++	++	++	++	+	++	+++	+
1:640	++	++	+++	+++	++	++	+++	++	++	++	++	++
1:1280	+++	++++	+++	++	++	+++	++	++	++	++	++++	+++
SERUM + N.S. (CONTROL)	++	++	++	+++	++	+++	++	+++	++	++	++	+++
Result	1:160	1:320	1:20	1:80	1:20	1:80	1:10	1:80	1:80	1:320	1:40	1:80

Table II represents the findings when pollen extracts were tested twenty-two months after they were prepared. Here again, it should be noted that the saline extracts deteriorated more than the other extracts. The glycerin extracts compare favorably in strength with the dried or cryochemed extracts, although the latter are somewhat stronger; the dried extract stored in the icebox is practically the same strength as the dried extract stored at room temperature.

A comparison of Tables I and II indicates further that there is a tendency for all extracts to lose strength, although the cryochemed extracts lose definitely less of their allergenic strength than do the saline and the glycerinized extracts.

Table III indicates the relative strength of other extracts prepared by the same technique in June, 1940, and tested twenty-two months later by the neutralization test. It is interesting to note that almost without exception the dried extract was not only as good as, but, as a matter of fact, stronger than the regular extract.

Just about the time this paper was being written we had the opportunity to see a young asthmatic boy who gave a clear-cut history of sensitivity to cabbage. He stated that ingestion of the smallest amount of cabbage, cooked or raw, or even the odor of cooking cabbage would induce in him an immediate attack of asthma. Because of this history, intradermal tests to the cabbage family (cabbage, cauliflower, broccoli, and Brussels sprouts) were performed with caution, using dilutions of 1:25 and 1:10. These yielded negative tests. Cabbage extract in a dilution of 1:5 gave a moderate minus reaction, while extracts of the other three vegetables of this group used in a dilution of 1:5 yielded doubtful or slight positive skin tests. It was thought that our failure to obtain more convincing positive reactions may have been due to the age of the extracts, and for this reason the tests were separated, employing freshly prepared (twenty-four-hour-old) saline extracts of each of these vegetables. The patient gave a moderate reaction to cabbage and cauliflower and a slight reaction to broccoli and Brussels sprouts.

Because fresh Brussels sprouts were not obtainable, frozen Birds Eye product of this vegetable was used in the preparation of this extract.

It was desired to observe whether a fresh cryochemed extract of these vegetables would be any stronger than the forty-eight-hour-old saline extract. For this purpose, a small amount of the saline extract was dried immediately after preparation. This dried extract was dissolved in distilled water and diluted 1:5 forty-eight hours later when it was employed for intradermal testing. Fortunately, the patient was tested first with only two of the vegetables; namely, cabbage and cauliflower. Within a few minutes after testing, the patient gave a marked intradermal reaction to both tests and developed a severe constitutional reaction. Intradermal tests performed the following day with freshly prepared 1:5 dilutions of broccoli and Brussels sprouts gave a moderate skin reaction to broccoli and a slight plus reaction to Brussels sprouts.

It was obvious, therefore, that the cryochemed extract of cabbage, cauliflower, and broccoli were, on the basis of the direct skin tests and clinical reaction, stronger than the saline extract which was prepared at the same time and certainly much more active than a twelve-month-old saline of the same substances. Some of these results should be interpreted in light of cross-antigenicity of members of the cabbage family.

In order to evaluate the quantitative differences in the strength of the two types of extracts, neutralization tests were carried out with a twenty-four-hour-old saline extract and a cryochemed extract which was seventy-two hours old. The comparative strength is indicated in Table IV. It is obvious that the saline extracts of these vegetables lost a great deal of their activity as compared with the dried extracts. Thus the strength of the saline cabbage extract is 1:10 as compared with the dried cabbage extract which was potent in a dilution of 1:160. Similarly, the saline cauliflower extract was potent in a dilution of 1:10 as compared with the dried extract which was potent in a dilution of 1:160. In other words, while the saline extract began to lose in strength almost immediately after preparation, the same extract, if dried right after its preparation, will give a satisfactory result when used for intradermal tests.

TABLE IV

NEUTRALIZATION TESTS INDICATING RELATIVE POTENCY OF SALINE (TWENTY-FOUR-HOUR-OLD) EXTRACTS AND DRIED EXTRACTS

REAGIN SERUM + EXTRACT TO BE TESTED IN DILUTIONS:	CABBAGE		CAULIFLOWER		BROCCOLI		BRUSSELS SPROUTS	
	SALINE	CRYO-CHEMED	SALINE	CRYO-CHEMED	SALINE	CRYO-CHEMED	SALINE	CRYO-CHEMED
Concentrate	0	0	0	0	0	0	±	0
1:10	±	0	±	0	±	0	+	+
1:20	+	0	+	0	+	0	+	+
1:40	+	0	+	0	+	+	++	+
1:80	+	0	+	0	++	+	+	++
1:160	++	+	++	±	++	++	++	+++
1:320	++	+	++	++	++	++	++	+++
1:640	++	++	++	++	+++	++	+++	+++
1:1280	+++	++	+++	++	+++	+++	+++	+++
SERUM + N. S. (CONTROL)	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.
Result	1:10	1:160	1:10	1:160	1:10	1:40	Conc.	1:10

pH OF EXTRACT

It is generally known that the pH of an extract is an important factor not only because acid-reacting extracts may produce nonspecific reactions, but also because these extracts have a tendency to lose their activity more quickly than the alkaline extracts. Hydrogen-ion concentration tests on various allergens, including fruits, such as oranges, and juicy vegetables, indicate no change in the pH after the dried extract is redissolved in distilled water. Desiccation apparently maintains the alkalinity of the extract.

Furthermore, no difficulty was encountered in desiccating by this method extracts of juicy fruits and vegetables. This is important, for

Tuft and Blumstein[14] have shown recently that these extracts lose their potency very rapidly following preparation.

BACTERIAL CONTAMINATION

Another factor to be considered in connection with the preservation of allergens in the dried state is that of bacterial contamination. It is known, of course, that freezing and drying will help avoid bacterial growth. For this reason, dried extracts which have been sterilized previously by seitzing continue to remain sterile for an indefinite period of time. The active principle of some substances, particularly in fruits and in vegetables, deteriorates easily with manipulation such as seitz filtration. In these cases, it may not always be necessary to subject these extracts to seitzing if one uses reasonable aseptic precautions in their preparation. These precautions should include the employment of sterile glassware and sterile extracting solutions. This would hold good only in exceptional situations and if the extract is to be used for the purpose of skin testing and not for the therapeutic purposes.

Bacterial colony counts were done on a number of extracts which were not seitzed, both before and after they were dried. While there appeared to be a slight drop in the count following cryocheming, this drop was insufficient to warrant the conclusion that the process of freezing has any bactericidal effect. Apparently the temperature of –20° C. produced in the cryochem apparatus is not sufficiently low to have any injurious effect on the bacteria present.

SUMMARY

1. The cryochem method of drying allergenic extracts is described. This method is practical and useful both in connection with pollen extracts and food extracts.

2. Evidence derived from direct skin tests, total protein nitrogen determinations, and neutralization tests is presented to indicate the comparative strength of standard saline extracts of some inhalants and some foods six months and twenty-two months after preparation.

3. This evidence indicates: (1) Saline extracts deteriorate rather quickly, in some instances almost within a few hours after preparation. (2) Glycerin extracts retain their strength but (a) are painful and (b) must usually be stored in the refrigerator. (3) Cryochemed extracts of the extracts examined retain their potency even when stored in a cool place but not necessarily in the refrigerator.

4. The pH of cryochemed extracts does not change.

5. There is reason to believe that drying of extracts helps avoid bacterial contamination.

REFERENCES

1. Martin, C. J.: J. Path. & Bact. **3**: 507, 1896.
2. Shackell, L. F.: Am. J. Physiol. **24**: 325, 1909.
3. Cragie, C. J.: Brit. J. Exper. Path. **12**: 75, 1931.

4. Elser, W. T., Thomas, R. A., and Steffen, G. T.: J. Immunol. **28**: 433, 1935.
5. Flosdorf, E. W., and Mudd, S.: J. Immunol. **29**: 389, 1935.
6. Ecker, E. E., and Pillemer, L.: Am. J. Pub. Health **28**: 1231, 1938.
7. Tuft, L., Wenger, L. J., and Frankel, J. J.: J. ALLERGY **10**: 27, 1938.
8. Rappaport, B. Z.: J. ALLERGY **5**: 13, 1933.
9. Cohen, Milton B.: J. ALLERGY **10**: 385, 1939.
10. Coca, A. F., and Grove, E. F.: J. Immunol. **10**: 445, 1925.
11. Cooke, R. A., Barnard, J. H., Hebald, S., and Stull, A.: J. Exper. Med. **62**: 733, 1935.
12. Cooke, R. A., Stull, A., Hebald, S., and Barnard, J. H.: J. ALLERGY **6**: 311, 1935.
13. Levine, P., and Coca, A. F.: J. Immunol. **11**: 411, 1926.
14. Tuft, L., and Blumstein, G.: J. ALLERGY. In press.

1004 MAY BUILDING

DISCUSSION

DR. MILTON B. COHEN.—There is one thing I think can be gotten out of several of the papers which we have had today, which was illustrated, I think, particularly by Dr. Criep's paper, and that is that we ought to handle our extracts, the materials we work with, gently. We do not know what we are working with. It is quite apparent we do not know the nature of house dust, and it is also apparent to most of us that we do not know much about the nature of any other extracts. Perhaps if we would learn to use such methods as Dr. Criep has advocated so that we could have the same material to work with a year, two years, or five years from now, we might get better results.

DR. BENNETT KRAFT, Indianapolis.—I would like to cite the case of a mother and child, both sensitive—the mother sensitive to fish, the child markedly sensitive to eggs and peas—both proved clinically. The child can handle frozen peas and frozen eggs without any symptoms of eczema. If we freeze an egg completely, then thaw it, prepare it any way we want, there are no symptoms. With the unfrozen egg, the child regularly has symptoms.

The mother has hives from fish. When the fish is frozen—and we have tried to substitute it so she does not know whether it is frozen—she does not have any symptoms.

If I am correct in what I thought I heard, according to Dr. Criep, these extracts retain their potency after freezing. My impression was, from these two cases, that freezing an allergenic substance destroys the allergenicity of the substance. Further experimentation along these lines might be of value.

DR. HOWARD OSGOOD, Buffalo, N. Y.—We have been using cryochemed ragweed extracts for about a year. We found that we have to be very careful to extract all the fats and lipids out of pollens before freezing and drying their extracts. Otherwise we get a gummy substance which will not go back into solution.

Another thing, we found a number of our extracts, on redissolving later, had a slight precipitate which could be centrifuged out. We were afraid we were losing some of our antigenicity.

However, we had already done nitrogen determinations before freezing and drying, and we repeated our nitrogen determinations after. There was no loss of nitrogen in this precipitate. We also did a few comparative tests as to the potency of the material before and after freezing, which showed no appreciable loss.

I should like to ask Dr. Criep about his experience with fat in the extracts and with the precipitation after freezing, drying, and redissolving.

DR. CRIEP.—My experience is similar to Dr. Osgood's; namely, if there is fat in the original material, the final product will be gummy. So it is necessary to remove the fat from the material.

Dr. Kraft understood me rightly; namely, freezing and drying extracts seems to preserve their potency.

DR. H. L. HUBER, Chicago.—I would like to answer the question raised by Dr. Kraft.

Several years ago I took ragweed pollen and tried to destroy or fragment it by freezing with liquid air. We froze it solidly—as many as twenty times, froze and thawed—and there was no disappearance of the activity.

FATALITIES AND CONSTITUTIONAL REACTIONS FOLLOWING THE USE OF PONTOCAINE

J. Warrick Thomas, M.D., Cleveland, Ohio, and
Meryl M. Fenton, M.D., Detroit, Mich.

THE question of pontocaine sensitivity as characterized by constitional reactions, some of which are fatal, is a timely subject. A review of our experience as well as a review of the literature seems to be practical and at the same time might obviate other unfortunate reactions. Nine cases have been collected of reactions which occurred during bronchoscopy, gastroscopy, dental extraction, and during the use of pontocaine in opththalmologic practice.

Fasselt[1] reported two marked reactions to pontocaine (2 per cent) used in anesthetizing the throat prior to the removal of foreign bodies by bronchoscopy. The symptoms disappeared spontaneously. Phillips and associates[2] reported a reaction occurring in a patient undergoing bronchoscopic examination. Approximately 5 c.c. of 0.5 and 1 per cent solutions combined were administered. Generalized tonic contractions developed, and the spasms were controlled by two injections of 2 c.c. of dial. Bronchoscopy was performed under pontocaine anesthesia three weeks later without untoward symptoms. Schoen[3] described three fatal reactions to topical anesthesia with pontocaine for bronchoscopy, two of the patients having been observed by other physicians. In the case reported by Cazzaniga, a total dose of somewhat less than 0.13 Gm. of pontocaine was used to anesthetize the throat. Severe convulsions developed a few seconds after the anesthesia, and the patient died approximately one-half hour later. In the case of Wagener 6 to 7 c.c. of a 2.5 per cent solution of pontocaine were applied to the larynx with a laryngeal syringe during a period of from six to eight minutes. Convulsions developed, and artificial respiration and cardiac and respiratory stimulants failed to prevent a fatal outcome. In Schoen's case 6 c.c. of a 2 per cent solution of pontocaine were sprayed into the larynx in order to anesthetize the bifurcation of the trachea, and 5.3 c.c. were dropped into the trachea by means of a laryngeal syringe. Tonic-clonic convulsions developed and extended over the whole body, including the jaw. Rigor mortis occurred.

In discussing a paper by Freeman,[4] Fitzgibbon mentioned having seen four serious reactions to the use of pontocaine anesthesia in gastroscopy. Approximately 13 c.c. of 2 per cent pontocaine hydrochloride were used in each patient. Hancock[5] reported two deaths from the use of pontocaine in gastroscopy. Both patients were extremely cachectic, suffering from carcinomatosis. Schindler[6] reported a similar death in a patient who also had multiple metastases of carcinoma.

Read at the Annual Meeting of the American Association for the Study of Allergy, Atlantic City, N. J., June 8, 1942.

Pfeiffer[7] reported a case of sensitivity to a 0.5 per cent solution of pontocaine, two drops of which were used in each eye for measurement of the ocular tension with a tonometer. A definitely positive reaction was obtained to a patch test with pontocaine. Schumacker[8] reported a case of sensitivity in an instillation of a 1 per cent pontocaine solution as anesthesia for the incision of two small chalazia and a small hordeolum on the upper lid of the right eye. An intradermal test with 0.1 c.c. of a 1 per cent solution of procaine was negative. A similar test with 0.5 per cent pontocaine produced a violent reaction. McAlpine and Berens[9] reported three cases of allergic blepharoconjunctivitis from pontocaine sensitivity and four cases of occupational dermatitis in ophthalmologists.

Videbech[10] reported three cases of pontocaine eczema in doctors, one of whom was himself. In his case skin tests with preparations such as novocaine and percaine gave no positive cutaneous reactions. However, a 2 per cent solution of pontocaine applied to the skin of the upper arm for twenty-four hours provoked a very lively reaction, whereas no such reaction was demonstrable in the skin of a nurse who served as a control. Avoidance of the use of pontocaine resulted in a disappearance of the eczema.

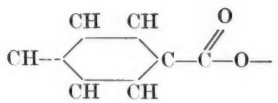
Cameron[11] reported a case of sensitivity to butyn and pontocaine, manifested by a marked dematitis of the fingers. Patch tests were positive to both butyn and pontocaine but were negative to cocaine, holocaine, and hematropin. Hollander [12] described a case of pontocaine contact dermatitis in an otorhinolaryngologist, and of the cutaneous tests made with the drugs and materials with which he came in contact, the test with pontocaine hydrochloride caused a decided reaction in twenty-four hours. Shimkin[13] reported a number of cases of professional eczema in oculists from the use of pontocaine.

In discussing Freeman's paper, Banks stated that he had not observed any ill effects from the use of pontocaine in gastroscopy, although he knew of nine reported deaths from its use in bronchoscopy and urethral instrumentation.

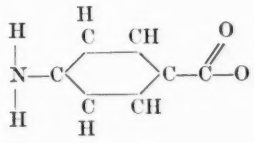
Throughout the literature there is a decided similarity in the character of the reactions reported.

It is interesting to consider the possible role of the chemical structure in influencing a sensitivity to a specific drug. In their review of the chemistry of local anesthesia, Hirschfelder and Bieter[15] for the purpose of simplification have divided the anesthetics into four groups, having the following radicals or structures:

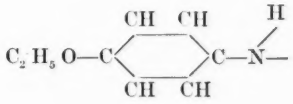
1. Benzoyl derivatives



2. Para-amino benzoyl group

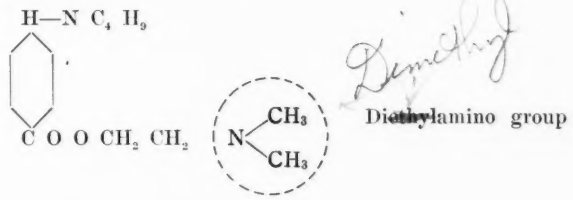


3. Phenetedin group

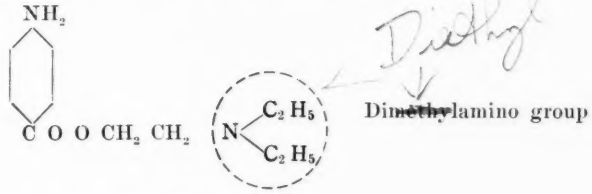


4. Quinoline group

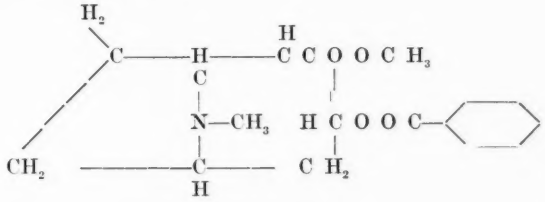
There is a similarity in the structural formulas of pontocaine and procaine since they contain the para-amina benzoyl group. The structural formula for pontocaine is:



The structural formula for procaine is:



Cocaine whose structure is:



contains the benzoyl group which differs from the para-amino benzoyl group and suggests the fact that when persons are sensitive to pontocaine or butyn, cocaine should be considered for anesthesia, as there is no frank group similarity. Furthermore, when a patient is sensitive to pontocaine, one should hesitate to use procaine since the two contain

the para-amino benzoyl group, and the difference in the two preparations is the grouping around the nitrogen atom dimethyl-amino group in the pontocaine and the diethyl-amino group in procaine. Certain patients may have multiple sensitivity to drugs containing different chemical groups as was the case in a patient with contact dermatitis and allergic conjunctivitis due to both pontocaine and nupercaine. The nupercaine contains the quinoline group.

CASE REPORTS

We are concerned primarily with the use of pontocaine as a local anesthetic in the preparation of patients for bronchoscopic and gastroscopic examinations. Also included is a case report in which the patient had previously reacted to a local anesthetic used for tooth extraction.

Death in an Asthmatic Patient Following the Use of Pontocaine in Bronchoscopic Preparation

CASE 1.—A 36-year-old man gave a history of asthma of several years' duration and of an occasional productive cough with wheezing. He was adrenalin-fast, and changes of climate had not relieved his symptoms. He also complained of allergic rhinitis but gave no history of drug sensitivity. Both antra were dark following a previous Caldwell-Luc operation, and there were characteristic allergic turbinates. Examination of the chest showed a percussion note hyperresonant throughout and a few scattered sonorous and sibilant râles. Bronchoscopy was advised, and the patient was admitted to the hospital.

Up until the morning of bronchoscopy, the only drug the patient received was epinephrine. At 9:30 A.M. he was given morphine, ⅙ gr. and atropine, 1/150 gr. At 10:45 A.M., 2 per cent pontocaine was sprayed into the throat which was repeated about 10:50 A.M. Following the second pontocaine spray, he was seized with a severe asthmatic attack. Five minims of adrenalin were given intramuscularly, which failed to relieve him. He rapidly went into shock and respiration ceased. Artificial respiration and intratracheal oxygen and carbon dioxide and coramine intravenously started respirations for a few minutes. At this time there were a few muscular twitchings, and 1 c.c. of sodium phenobarbital was given intravenously. The convulsion stopped, and respiration again ceased. The heart went into fibrillation and stopped at 11:20 A.M. One-half of a cubic centimeter of adrenalin intracardially, artificial respiration, oxygen, and carbon dioxide failed to revive him.

Comment.—This patient had been seen in a number of clinics without obtaining relief and had requested that all examinations including bronchoscopy be carried out. There was no contraindication to the use of pontocaine from this patient's history other than asthma. General precautions were taken, and all possible measures were used to revive the patient without avail.

Constitutional Reaction to Pontocaine in a Presumably Nonallergic Individual

CASE 2.—A 37-year-old housewife gave a history of cough and loss of weight over a period of one year. A tentative diagnosis was made of pulmonary tubercu-

losis and possibly tracheobronchial tuberculosis. There was no history of allergy. Accordingly bronchoscopic examination was considered.

At 1:40 P.M. in preparation for bronchoscopy the patient received morphine, ⅙ gr., and atropine, 1/150 gr., by hypodermic. At 2:00 P.M. 2 per cent pontocaine was administered as local anesthesia. She had received about 5 c.c. when she became faint and went into a generalized convulsive seizure with frothing at the mouth. A generalized tonic convulsion developed with cyanosis and weak pulse. This subsided, and respirations and pulse returned, to be followed by two similar attacks with no return of pulse or respiration after the second. She was given artificial respiration, oxygen, and carbon dioxide, followed by caffeine sodium benzoate, 7½ gr., and adrenalin, 10 minims, and later by coramine, 1 c.c., and adrenalin, 15 minims, intracardially. There was no response. Respiration ceased.

Comment.—This patient belongs to that group of unfortunate patients in whom the physician had taken general precautions and had used all means at hand without avail when the reaction occurred.

Fig. 1.—Case 3.

Constitutional Reaction to Pontocaine in Patient With Suspected Bronchiectasis

CASE 3.—A 48-year-old woman gave a history of recurrent attacks of influenza and of pneumonia, Type III, in 1939 which had been treated with sulfapyridine. She had a cough productive of thick yellow sputum. Previous x-rays and sputum examination were negative for tuberculosis. The patient was referred for lipiodol instillation with a diagnosis of probable bronchiectasis.

She was admitted to the clinic for lipiodol instillation and lung mapping. Following the administration of seconal, 1½ gr., and pontocaine spray, 2 per cent to the

throat and 3 c.c. intratracheally, the patient had a severe drug reaction. She developed feeble respiration, cyanosis, a weak pulse, and a clonic convulsion, which was not true tetany. Three-fourths of a cubic centimeter of adrenalin was given intramuscularly, and oxygen was administered. The patient returned to consciousness after about thirty minutes. Severe bronchial congestion and coughing followed the reactions.

The patient was referred to the allergy department for investigation of the suspected drug sensitivity. About eight or nine years previously the patient had developed a generalized trembling or shaking seven to ten minutes after the injection for the extraction of a tooth. The reaction lasted fifteen minutes and caused the operator definite concern. No personal or familial history of allergy was elicited.

A patch test to seconal was questionably positive, and that to pontocaine revealed a 2+ reaction. The test with cocaine and the control were negative.

Comment.—Because of the 2+ patch test skin reaction to pontocaine and only a questionable reaction to seconal, we believe that this was a true pontocaine reaction. Some workers believe that if a barbiturate derivative is given in preparation for anesthesia, the possibility of a pontocaine reaction is lessened. Possibly if a very large dose of seconal had been given in this case, the patient might not have had the reaction. Twitching is not characteristic of a seconal reaction, and a similar drug is given to control such convulsive tendencies in the treatment of pontocaine reactions.

Fig. 2.—Case 4.

Constitutional Reaction in a Patient With Allergic Bronchitis and Questionable Bronchiectasis

CASE 4.—A 60-year-old patient had had a cough for five years which had increased in severity during the previous two weeks. The cough was productive of a clear mucoid sputum which at times became mucopurulent. Sputum examinations for tuberculosis were negative, and x-rays of the chest were normal. Sinus x-rays

revealed some thickened membrane. Physical examination revealed that the lungs were slightly hyperresonant. Slight moist scattered râles were found over the left base posteriorly.

Early bronchiectasis was suspected, and as the patient showed no appreciable benefit from allergic treatment, bronchoscopy and lung mapping were advised. While having pontocaine applied to the larynx and vocal cord, the patient had a severe convulsive seizure and became cyanotic. Oxygen-carbon dioxide mixture was given, and 2 c.c. adrenalin were injected intracardially. Four grains of sodium luminal were also given intravenously.

A patch test to pontocaine was positive and produced a generalized reaction which required her to go to bed. The patch test to cocaine was negative. Later bronchoscopic examination with cocaine was performed without incident. Cocaine had been used frequently in the nose of this patient without trouble.

Comment.—This patient did not have a true asthma but an allergic bronchitis. Heretofore I have seen no report in the literature of a constitutional reaction in allergic bronchitis or any warning as to possible drug reactions as in the case of bronchial asthma. All allergic patients, however, are potential candidates for drug reactions.

Pontocaine Reaction in a Patient With History of Constitutional Reaction at Time of Dental Extraction

CASE 5.—A 55-year-old patient complained of attacks of abdominal pain which was not localized. Five weeks previously the pain had localized over the gall bladder with spasm and marked rigidity. Occasionally, complete relief had been obtained from morphine. Nasal obstruction and some sneezing accompanied the attacks. Gall bladder disease was suspected, and x-ray revealed an almost nonfunctioning gall bladder.

Because of a suspected allergic factor in the case, the patient was referred for allergy study. At that time patients were being routinely patch tested for pontocaine sensitivity. When a plus reaction was obtained, the patient reported after further questioning that he had had a constitutional reaction after a tooth extraction. Since that time he has refused all local anesthesia for extractions.

Comment.—As this case indicates, patients frequently do not report a drug sensitivity when questioned routinely, and it was only after a positive patch test with pontocaine that the patient admitted having had a former reaction to a local anesthetic. This case also points out the similarity between pontocaine and procaine, which no doubt was used as a local anesthetic by the dentist, and the patient may have been sensitized at the time of his tooth extraction.

Pontocaine Reaction in Postoperative Gastrectomy at time of Gastroscopy Preparation

CASE 6.—The patient, a 58-year-old man, complained of epigastric pain, loss of appetite, vomiting, and loss of eighteen pounds in weight. The emesis was dark brown to black in color, and the stools were black. A diagnosis of a probable bleeding ulcer was made, and cancer had to be ruled out. Further study revealed a definitely hard neoplastic mass, and x-ray revealed a neoplastic process along the lesser curvature.

The patient was admitted to the hospital, and a gastric resection was performed. Approximately two months after discharge the patient returned for progress gas-

troscopy study. He received the usual preparation of 2 gr. of sodium phenobarbital and 1/150 gr. of atropine subcutaneously. Approximately forty minutes later he gargled with 2 per cent pontocaine which was followed a few minutes later by a 5 c.c. spray which was repeated in ten or fifteen minutes. Fifteen minutes later the patient became stuporous, and there were clonic convulsions of the arm and leg. There were some tearing from the eyes, drooling and grinding of teeth. The expression of the facies was fixed, and the skin was cold and clammy. The lips became markedly cyanotic, and the hands were a deep blue color. The pulse was of good quality and regular. The reaction lasted for some ten minutes with the muscular contractions gradually diminishing, but the stupor persisted for another ten or fifteen minutes. A reaction to pontocaine was suspected, and patch tests with cocaine, pontocaine, and phenobarbital were all negative. A subsequent gastroscopy was performed without incident, using pontocaine without phenobarbital.

Comment.—This reaction may very well have been due to the absorption of large amounts of pontocaine. I question a phenobarbital reaction since the patient had clonic convulsions of certain groups of muscles. We would certainly question the desirability of proceeding with the use of pontocaine a second time after a suspected reaction.

Pontocaine Death Following Preparation for Gastroscopy

CASE 7.—The patient was admitted to Eloise Hospital for diagnostic procedures with reference to a ruptured ulcer of the stomach. Morphine sulfate, 1/4 gr., and atropine, 1/150, gr., were given in preparation for gastroscopy. Following the second tubing of the pharynx and hypopharynx with 5 c.c. of 2 per cent pontocaine solution, the patient was in good condition for a period of about eight or ten minutes. He suddenly became convulsive and pulseless and had jerky respiration. He was given 3.5 c.c. of sodium amytal intramuscularly and 3.5 c.c. intravenously. Artificial respiration was instituted, adrenalin was given intracardially, and he had metrazol. The patient showed no response, and pulmotor stimulation was without avail. This patient had a complete autopsy, and it was the impression of the pathologist that the gross tentative diagnosis was of carcinoma of the stomach with metastasis to the liver; acute passive hyperemia of viscera; dilatation of right ventricle and auricle; benign subcervical enlargement of prostate; fibrous pleural and peritoneal adhesions.

Comment.—This case has to be considered as a death due to pontocaine, and the possible explanation of the mode of entry of the drug into the circulation is that of trauma, since the neoplasm presented a hollowed-out ulcer crater in the midportion overlying the greater curvature.

Mild Constitutional Reaction to Pontocaine in a Cardiac Patient Following Preparation for Gastroscopy

CASE 8.—The patient was admitted to Eloise Hospital with the symptoms of a chronic cholecystitis, arteriosclerotic heart disease, and general arteriosclerosis. In preparation for gastroscopy, nembutal, 1½ gr., at 8 A.M., codeine, 1 gr., and atropine 1/100 gr., at 9 A.M. were administered. Pontocaine spray and mouth preparation was made at 9:15 A.M.; pontocaine, 5 c.c., by esophageal tube at 9:25 A.M. was repeated at 9:35 A.M. The patient began to have twitchings of the extremities, more marked on the right side, stiffening of the whole body, and wandering of the

eyeballs. The pupils reacted to light. There was no response to questioning. The pulse was strong and respirations deep. The patient was fully conscious and responded to questioning at 10:30 A.M.

Comment.—Nystagmus has been reported as a part of constitutional reactions by Fasselt and Freeman, which is a part of the general picture of the reaction with the muscular twitchings. The patient had nembutal, 1½ gr., as a part of the preparation for gastroscopy, and it is interesting to surmise that had the patient not had this drug, the reaction might have been more serious. In retrospect, this man's cardiac condition may have been a contraindication to the use of pontocaine.

Constitutional Reaction to Pontocaine Possibly Lessened by Previous Use of Nembutal as Part of Preparation for Gastroscopy

CASE 9.—The patient, a chronic alcoholic, was admitted to the hospital with symptoms of having had acute gastroenteritis of unknown etiology. In preparation for gastroscopy, nembutal was given at 8 A.M., a pontocaine spray at 9:25 A.M., pontocaine mouth preparation at 9:30 A.M., pontocaine, 5 c.c., by esophageal tube at 9:35 A.M., which was repeated at 9:40 A.M. At 9:50 A.M. a convulsive seizure started with rolling of the eyes, stiffening of the neck, back, arms, and legs. The eyes tended to deviate to the right. The pupils were dilated, equal, and reacted to light. There was clamping of the jaws and drooling. The pulse was strong and respirations deep. Sodium amytal, 3¾ to 4 gr., was given intravenously at 10 A.M. At 10:10 A.M. he started to react by gagging and coughing.

Comment.—This patient also had nembutal as a part of this preparation, and only after the last pontocaine application did the constitutional reaction develop. The use of intravenous sodium amytal further emphasizes the importance of such drugs in the control of the reactions. This patient did receive a rather large amount of the drug.

DISCUSSION

Specimens of the 2 per cent pontocaine hydrochloride, which was used in the aforementioned examinations, was submitted to the Committee on Pharmacology and Chemistry of the American Medical Association and were reported to be pure in form. Accordingly these reactions seem to be purely those of a drug sensitivity and not due to any impurities in the drug.

A communication from the manufacturer,[16] enclosing a folder of descriptive literature on pontocaine hydrochloride and surface anesthesia, recommends that when sprayed into the trachea, the amount of pontocaine hydrochloride should not exceed 20 mg. (1 c.c. of a 2 per cent solution or a corresponding volume of more dilute solution). It is suggested that extreme caution is imperative when any local anesthetic, even in relatively small amounts, is injected into the traumatized urethra, or under conditions in which trauma is likely to occur. In the booklet, however, no notation is made of death having resulted from the use of this drug.

Some of these patients had one type of preoperative preparation and others a different type with practically comparable results. Some preparations included the use of seconal or phenobarbital or like drugs, and others did not.

The use of pontocaine in cases for bronchoscopy has been discontinued at the Cleveland Clinic, and the present preparation includes 1½ gr. of nembutal or seconal one to one and one-half hours prior to examination. In addition, codeine, 1 gr., and atropine, 1/150 gr., are given one-half hour before examination. On occasion, morphine, ⅛ to ⅙ gr., has been used in place of codeine, but since there has been some nausea or vomiting as a result of this drug, we prefer not to use it as it frequently interferes with the examination. The throat is sprayed with 4 per cent cocaine two or three times, and 10 per cent cocaine is applied by swab to the pharynx and pyriform sinuses. About 1 c.c. of 2 per cent cocaine is given intratracheally. Approximately the same procedure was followed at the time pontocaine was being used in place of various strengths of cocaine solution.

TABLE I

RESULTS OF 201 PATCH TESTS WITH COCAINE, PONTOCAINE, AND CONTROLS IN ALLERGIC PATIENTS

Patients patch tested	201
Patients with + or ± reactions to cocaine	2
Patients with + or ± reactions to pontocaine	20
Patients with suggestive reactions to pontocaine	3
Positive control tests	0
Patients confirming reaction to pontocaine	4

Patch tests with pontocaine and cocaine were made in a number of allergic individuals. The results are outlined in Table I. Out of 201 patients tested two patients showed either + or ± reactions to cocaine, which is less than 0.5 per cent. However, out of this group, twenty + or ± reactions were obtained to pontocaine, or over 9 per cent possible sensitivities to this drug as evidenced by patch tests.

TABLE II

CORRELATION OF PATCH TESTS WITH CASE REPORTS

Total cases reported	9
Patch tests	5
+ or ± reactions to pontocaine	4
Negative reactions	1

We were able to confirm the constitutional reactions by positive patch tests in two of the patients undergoing bronchoscopy. In one patient with a reaction during gastroscopy (apparently from overdosage), no positive patch test was obtained. Pontocaine was used again later, and gastroscopy was carried out without a reaction. None of the Detroit patients had patch tests, and none of the patients who died had patch tests (Table II).

One of us (M. M. F.) made forty-nine control tests with pontocaine in ½ per cent, 1 per cent, and 2 per cent dilutions. There were six ± reactions, and two 1+ reactions. Two of the group showing ± reactions also demonstrated reaction to the ½ and 1 per cent pontocaine as well as to the 2 per cent dilution.

When reactions occur, there are several drugs that have to be considered as emergency measures. In most instances we have employed epinephrine, both intramuscularly and intracardially; caffeine sodiobenzoate has been used to stimulate the respiration along with caffeine. To control the muscular twitching and convulsive movements, we have used sodium phenobarbital intravenously. The dosage of each of these is variable, depending upon the patient's condition and the drug effect desired. Oxygen-carbon dioxide mixtures have been employed in stimulating respiration. It is desirable to have on hand picrotoxin, should the patient be given too much of the sodium phenobarbital. Other drugs or procedures may be used according to personal preference.

There are several precautions to be considered before giving a patient pontocaine; namely, the question of a previous allergy including bronchial asthma; emaciation; cardiac or thyroid complications; endocrine disorders; and in cases where there are open wounds or traumatized areas. Overdosage of the drug should always be kept in mind, and emergency measures and facilities should be available for immediate use. Some investigators have discussed the question of patch tests prior to the use of pontocaine, although this is a matter of opinion and depends upon the individual case. If there is any doubt about the history, patch tests should be considered. However, they are not 100 per cent correct, and there may be false positive and false negative reactions.

SUMMARY

1. The literature to date has been reviewed and the significant data included in this report. Structural formulas have been outlined with the suggestion that certain chemical groups might have a bearing upon the selection of drugs for local anesthesia. Further it suggests that there might be a certain group structure that may influence a reaction.

2. Seven cases of reactions following the use of pontocaine have been reported, including three deaths. Four reactions occurred from the use of the drug in bronchoscopic preparation or examination. Three reactions followed the use of the drug in preparation for gastroscopic examination.

3. Suggestions for control of reactions are outlined as well as precautions to be taken when pontocaine is used. Patch tests studies were made on known allergic patients and pontocaine-sensitive patients. Results of this study are reported.

4. Our experience can certainly substantiate the experience of other workers as far as the character of the reaction and the correlation of

symptoms in certain groups of individuals; i.e., the allergic patients (as in bronchial asthma) and in patients without a definite history of allergy, such as emaciated individuals.

REFERENCES

1. Fasselt, H.: Zwischenfälle bei Pantocainoberflächenanästhesie, Arch. f. Ohren-, Nasen- u. Kehlkopfh. **146:** 217, 1939.
2. Phillips, F. J., Congleton, V. L., and Tuttle, W.: Pontocaine Hydrochloride Reaction Relieved by Dial, Anesth. & Analg. **20:** 233, 1941.
3. Schoen, F.: Tödliche medizinale Vergtflungen durch Pantocain, Wien. klin. Wchnschr. **52:** 505, 1939.
4. Freeman, E. B.: Gastroscopic Study Compared With Other Methods of Diagnosis in Gastric Lesions, J. A. M. A. **112:** 217, 1939.
5. Hancock, P. E. T.: Discussion on Practical Application of Gastroscopy, Proc. Roy. Soc. Med. **32:** 538, 1939.
6. Schindler, R.: Results of Questionnaire on Fatalities in Gastroscopy, Am. J. Digest. Dis. **7:** 293, 1940.
7. Pfeiffer, R. L.: Hypersensitivity to Pontocaine; Report of Case, Arch. Ophth. **18:** 62, 1937.
8. Shumacker, H. B., Jr.: Reactions to Local Anesthetic Agents; Clinical Report, Surgery **10:** 134, 1941.
9. McAlpine, P. T., and Berens, C.: Allergic Dermatitis and Blepharoconjunctivitis Caused by Pontocaine, Am. J. Ophth. **25:** 206, 1942.
10. Videbech, H.: Eczema in Doctor Caused by Handling Pontocaine, Ugesk. f. laeger **98:** 278, 1936.
11. Cameron, O. J.: Skin Eruptions Due to Nupercain and Pantocain, Nebraska M. J. **22:** 229, 1937.
12. Hollander, L.: Contact Dermatitis Due to Pontocaine Hydrochloride, Arch. Dermat. & Syph. **40:** 92, 1939.
13. Shimkin, N.: La pantocaïne, cause d'eczema professionnel chez l'oculiste, Ann. d'ocul. **176:** 198, 1939.
14. Jackson, C. L., and McReynolds, G. S., Jr.: Anesthesia for Peroral Endoscopy, Ann. Otol., Rhin. & Laryng. **49:** 1048, 1940.
15. Hirschfelder, A. D., and Bieter, R. N.: Local Anesthetics, Physiol. Rev. **12:** 190, 1932.
16. Zander, E.: Personal communication, Winthrop Chemical Company, New York City.

DISCUSSION

PAUL M. MOORE.—Doctors Thomas and Fenton have presented an extremely important subject and have covered it thoroughly and to the point. This is an important subject not only to the endoscopist but to allergists, who see the type of patient who is most likely to have a severe reaction. Even though the patch test is not 100 per cent reliable, as Thomas and Fenton have pointed out, it is an additional safe-guard and will pick up some of these cases before the anesthesia is administered. I believe that the markedly allergic individual at least should have a patch test before he is referred for bronchoscopy.

While these reactions do not occur frequently, when they do occur they come with devastating swiftness and the results may be disastrous. I had used 2 per cent pontocaine exclusively in all of my endoscopic work for six years before I encountered a reaction of any kind. This was one of the fatalities reported by Dr. Thomas. Within a period of two months after that we had two more reactions, one of which was very close to being fatal. This patient was subsequently bronchoscoped with cocaine anesthesia after patch tests had been made, which showed her to be sensitive to pontocaine but not to cocaine.

Until that time I had been using 2 per cent pontocaine as a spray to the throat and following this with application on a cotton swab to

the pyriform sinuses; I then dropped a small amount intracheally. Although a sensitized individual will react to small amounts of the specific allergen, I cannot help but feel that the quantity and method of injecting intratracheally may be very important. If a fair amount is injected in one quick shot, it may all go down one bronchus and into an alveolus. If this occurs, it is absorbed from the alveolus very quickly and the effect on the patient is the same as though a like quantity had been given intravenously.

In the preoperative preparation of the patient, it is important that he receive a barbiturate, for this tends to prevent or reduce the severity of reactions to the local anesthetics. We use nembutal or seconal, 1½ gr., given one to one and one-half hours before the examination. One-half hour later a hypodermic of codeine sulfate, 1 gr., and atropine, $\frac{1}{150}$ gr., is given. For some years we used morphine but so many patients were encountered who were nauseated by the drug that we have largely discarded it. For the local anesthesia we now use 4 per cent cocaine as a spray in the pharynx, 20 per cent cocaine applied to the pyriform sinuses with a cotton swab, and drop 2 per cent cocaine intratracheally.

Our reactions were almost all of the clonic-tonic convulsive type, simulating a grand mal epileptic seizure but followed by actual or threatened cardiac and respiratory failure.

One should constantly have in the examining room an emergency tray fully equipped with hypodermic and intravenous syringes and needles. There should be a barbiturate suitable for intravenous injection to control the convulsion and counteract the drug. This is of the utmost importance and should be administered immediately. We have used the intravenous preparation of sodium phenobarbital. Epinephrine, caffeine sodium benzoate, and other stimulants should be included. Picrotoxin should be available in case too much barbiturate is given, but the time element here is not so urgent. Oxygen and oxygen-carbon dioxide should be handy and artificial respiration used as indicated. All of these things should be ready for immediate use, because when a reaction occurs one must act swiftly and surely, for it may be a matter of split seconds between recovery and death.

Dr. Leon Unger, Chicago.—In Dr. Thomas and Dr. Fenton's paper, they itemized the patients they reported. I noticed that three deaths were in patients in whom morphine had been given prior to the gastroscopy or bronchoscopy. The first two deaths were in asthmatic patients, Cases 1 and 2. Case 6 was a patient with a gastrointestinal rupture, and morphine and atropine had been given before the spray was used. This, of course, brings up the question of using morphine in allergic individuals, a very questionable practice, and one which I believe is contraindicated.

In the case of ruptured ulcer evidently nonallergic, does the combination of morphine and pontocaine cause harm which would not occur from either drug alone? We know morphine acts by depressing the respiratory center and by depressing the cough reflex, and also, perhaps, by anaphylaxis or allergy.

In any case, asthmatic patients should not receive morphine preparatory to bronchoscopy.

I noticed that Dr. Moore states that they have discontinued the use of morphine. I think that is a very wise precaution. Codeine certainly

can be used without any fatality. It will be noted that the patients in whom morphine was not used had constitutional reactions but all recovered.

Dr. Francis M. Rackemann, Boston.—I should like to ask a question for my own information. Has Dr. Thomas or, indeed, anybody, had experience with a very simple test for drug allergy; namely, putting some of the drug on the tip of the tongue and holding it there for five or ten minutes and spitting it out again? The idea is that the drug will create a local test reaction in situ.

I read about this some place but I cannot remember where. I have no experience with it, but I just wondered if anybody else knew about it.

Dr. Ralph H. Spangler, Philadelphia.—I was interested in the essayists' and the discusser's question of the convulsive manifestations. I have had one patient with epilepsy who has developed bronchiectasis, which those in Dr. Jackson's clinic at Temple University seemed to think was more or less the result of the severe epileptic spasms he had had. He has been treated at that clinic for about three years. Preceding his treatment for bronchiectasis in which pontcaine was used, no morphine, he has always had some sodium amytal. One injection has been given at the clinic a couple of hours before they have proceeded to give the treatment for the bronchiectasis. I think this may be of interest as a matter of precaution if it is necessary to do any bronchoscopic work on an epileptic patient.

Dr. Philip Blank, Pittsburgh.—In answer to Dr. Rackemann about the patch test for drugs, at Fort Eustis we have been having a few reactions from sulfathiazole and the sulfonamide drugs.

We have found that the simplest method for testing is to have the patient hold a tablet of the drug on the buccal mucosa for ten minutes and to make the reading in twenty-four hours. We had a rather unusual happening just before I came here. A patient was referred in for testing to the sulfonamide drugs. He was tested and in three minutes developed marked itching and burning at the site of the test and in twenty-four hours developed an ulcerative stomatitis with marked necrosis at the test site. The entire mouth became covered with ulcers and blebs necessitating hospitalization. He had had a terrific reaction from the use of sulfathiazole, characterized by marked gastrointestinal symptoms and an exfoliative dermatitis.

Concerning pontocaine, we have been using this as a spinal anesthetic. I wish to ask the doctor if he has had reports of any fatalities or reactions. We had a near accident. One man referred for hemorrhoidectomy was terrifically sensitive to sodium amytal and was given sodium amytal the night before to quiet him for his operation and developed severe urticaria and angioneurotic edema. He then was tested for the various spinal anesthetics by patch test, was markedly sensitive to them all. He was given an inhalant anesthetic uneventfully.

Dr. Thomas, Cleveland.—Apparently Dr. Unger misunderstood me in the fact that the second case was a nonallergic individual with a diagnosis of previous pulmonary tuberculosis and tubercular tracheitis.

The first case was an asthmatic patient whom I referred for bronchoscopy. I did not order the preoperative drugs, which may have been my fault. We felt, though, that since the patient died approximately

at 11:20 or at 11 o'clock and received the morphine at 9 o'clock, we could pretty well rule out morphine, since death occurred after the second spraying of the throat. He went into an acute paroxysm of asthma.

I give no morphine to allergic individuals of any description.

In reply to Dr. Rackemann's question of mucous membrane tests, I think this work was carried on by Dr. Duke where he put an aspirin tablet on the tip of the tongue in testing a patient for aspirin sensitivity. I have tested patients with karaya gum as used in some of the bulk-producing catharties. I have one patient who did show a definite congestion and swelling of the buccal mucous membrane inside of the lip. In one other case of sensitivity to karaya gum, we applied the preparation to the rectal mucosa, and an area of edema, redness, and congestion was present for over twenty-four hours.

I was interested to learn of the experience of the doctor from Virginia relative to the sulfonamide drug used in a mucous membrane test. I think that that should be a warning for us to proceed very cautiously in suspected patients.

In the cases that were presented, no history could be elicited of epilepsy. Certain of these cases received a barbituric acid, nembutal, or something of that type, to obviate the reaction to pontocaine. This is not 100 per cent. It is a nice precaution to take, but you cannot put too much faith in it. We have given these patients such preoperative preparation and they still had reactions.

KOCHIA SCOPARIA AS A FACTOR IN INHALANT ALLERGY

OREN C. DURHAM
NORTH CHICAGO, ILL.

THE recent rapid spread of the plant known as burning bush, fireball, firebush, Mexican fireweed, and summer cypress has suggested the need for an audit covering its present geographic range, its seasonal behavior, the incidence and toxicity of its pollen, as well as an inquiry into the cause of its aggression and, if possible, a forecast regarding the outcome of its clash with ragweed. The inquiry has been facilitated by the accumulated statistics from our widespread network of United States Weather Bureau air-sampling stations and particularly by the distinct character of the pollen granules of this species.

Kochia scoparia is an erect, densely bushy, much-branched annual one to five feet high with narrow linear leaves and inconspicuous axillary flowers. The stems and leaves are green or pink during the spring and summer, but at maturity often turn to a brilliant red or purple. Under favorable growing conditions the typical plant is usually globular or ellipsoidal in shape. As a weed it has a strong resemblance to Russian thistle with which it is frequently associated as a formidable competitor. Both plants are known as tumbleweeds and both are introduced agricultural pests that have literally gone with the wind in the Dust Bowl area. The Russian thistle invasion began about sixty-five years ago, preceding the burning bush invasion by at least three decades. Russian thistle can be used for hay if cultivated and cured when tender, but burning bush because of its saponin content is unfit for forage and is regarded by agriculturists as poisonous.

Whether the coarse wild form of kochia which is now rampant in Iowa, Nebraska, and Colorado and threatening adjacent states is a reversion from the cultivated variety, *Kochia trichophylla (Kochia scoparia trichophylla)*, which is commonly used as a decorative plant under the name summer cypress, or whether it is a comparatively recent imported strain of unusual vigor, authorities do not agree. Under the description of *Kochia scoparia,* Georgia[1] says, "Wherever this plant is established as a weed it has usually first been cultivated in gardens for its bright coloring in autumn." Kinch[2] says that "the ornamental summer cypress is nothing more than a cultivated form of kochia, which will revert back to the wild form in a few years if it is allowed to do so." At any rate, neither the original escape nor the seeds causing the present scourge came from Mexico, as the misnomer "Mexican fireweed" would seem to indicate, but rather from southern Europe or Asia.

From the Biological Laboratories of Abbott Laboratories, North Chicago.
Read at the Annual Meeting of the American Association for the Study of Allergy, June 8, 1942.

Fig. 1.—Burning bush (*Kochia scoparia*). Cultivated plants in the foreground. Wild growths on the opposite side of the street, along the curb, and in the vacant lot.

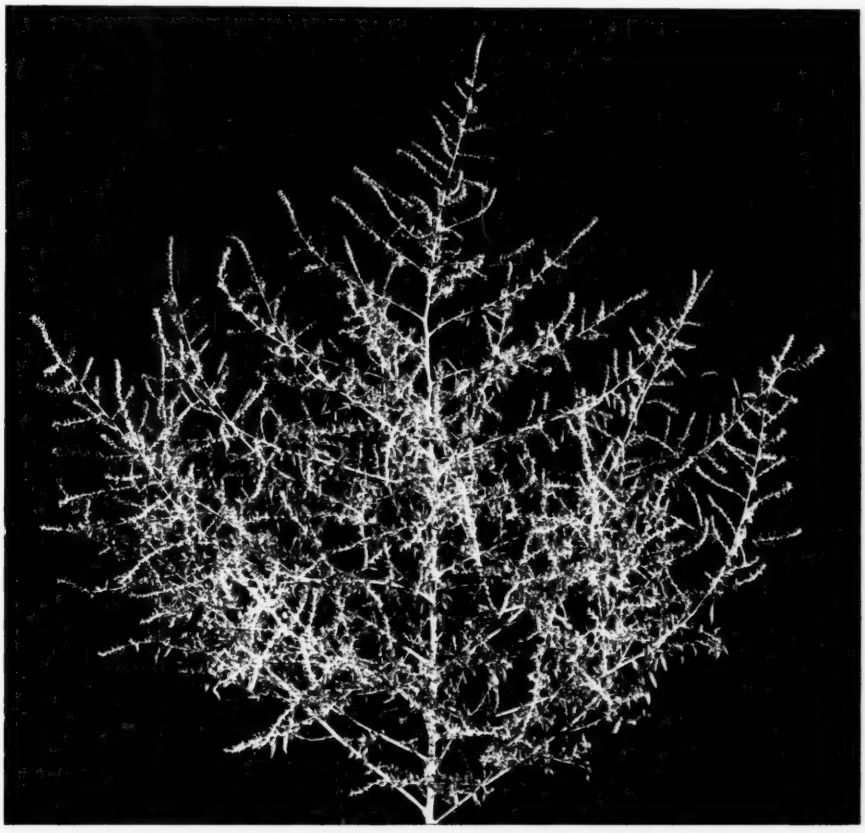


Fig. 2.—Burning bush (*Kochia scoparia*).

Sometime before 1753 Linne classified the plant as a true goosefoot and named it *Chenopodium scoparia*. Some years later Crantz declared it to be a saltbush and suggested changing the name to *Atriplex scoparia*. Even after Schrader in 1809 had placed it in the new genus, *Kochia*, another systematist insisted on calling it *Salsola scoparia*, a Russian thistle without thistles. Thus regardless of the genus to which it has been assigned at different times, it has always retained the specific name given it by Linne and has always remained in the chenopod family (*Chenopodiaceae*). The relationship of this plant to other common North American members of the order *Chenopodiales*, as shown in Table I (Standley[3]), is of prime importance in its evaluation as a cause of inhalant allergy.

TABLE I

NORTH AMERICAN RELATIVES OF KOCHIA SCOPARIA

ORDER	FAMILY	GENUS	SPECIES	COMMON NAME
Chenopodiales (11 families)	Chenopodiaceae (26 genera, 222 spp.)	Beta (1 sp.)	B. vulgaris	Beet
		Chenopodium (52 spp.)	C. album	Lamb's-quarters
		Spinacia (1 sp.)	S. oleracea	Spinach
		Atriplex (103 spp.)	A. wrightii	Annual saltbush
		Kochia (4 spp.)	**K. scoparia**	**Burning bush**
		Salsola (2 spp.)	S. pestifer	Russian thistle
	Amaranthaceae (21 genera, 166 spp.)	Amaranthus (42 spp.)	A. retroflexus	Pigweed
			A. spinosus	Spiny amaranth
			A. palmeri	Palmer's amaranth
		Acnida (8 spp.)	A. tamariscina	Western water hemp

The genus *Kochia* was introduced into the literature of allergy in 1918 by Selfridge[4] who included the two perennial species, *Kochia americana* and *Kochia californica*, in a list of "possible hay fever plants of secondary importance" in the western states. Four years later, Mullin,[5] working in Colorado Springs, observed that *Kochia scoparia* "often grows in gardens, has escaped abundantly at Pueblo and is an important cause of hay fever, while in Colorado Springs less than fifty miles north it is so far known in but one garden from which it has escaped into the adjacent railroad right-of-way." In 1926 Waring and Pope[6] listed the pollen as a "very, very common cause of hay fever" in central Colorado and the plant as "very common in all vacant lots and along roads and ditches" not only in Pueblo and Denver, but in Colorado Springs as well. At that time it was present in the packing house

district of Kansas City but not elsewhere in the area. Nor was it present in significant amounts in any of the large cities of the central and eastern states.

In 1929 a Nebraska Department of Agriculture weed bulletin[7] reported burning bush as a serious weed pest in Lincoln County. This is the very county where (at North Platte) I first found any high atmospheric concentration of kochia pollen, and that only a year after the state bulletin was issued. Ten years later, in the 1939 revision of *Nebraska Weeds*,[2] there appeared this interesting comment: "In the last fifteen years kochia has spread like wildfire over the whole state." By that time it was an abundant vacant lot weed at Omaha, though the amount of pollen in the air in Omaha was less than one-tenth of what it is now. Under a 1941 date the Kansas State Board of Agriculture[8] reported it as established in the western two-thirds of Kansas and "spreading rapidly" throughout the state. It has now reached central Iowa as shown by the results of slide counts at Des Moines and Ames[9] during the last two and four years, respectively.

Systematic surveys of Chicago in 1925[10] and again in 1933[11] did not reveal this weed, but last year I was surprised to find that the plant is firmly and widely established in the south section of the city, particularly in the stockyard area, where in many waste places and vacant lots it has crowded out ragweed and all other competitors. I was not aware of its advent in Milwaukee until the pollens began appearing on the weather bureau slides. A subsequent local inspection showed an abundance of the weeds over a considerable area. In many vacant lots it is decidedly dominant. The count at Minneapolis though still low has increased fivefold in five years. The weeds are also well established in Detroit and have gained a foothold in St. Louis[12] and Indianapolis but not in Cleveland. East of the Mississippi the present infestation seems to be confined largely if not wholly to urban centers.

The highest totals for seasonal pollen incidence are still the North Platte records taken in 1932 and 1933. Whether the count has continued to increase since then I do not know. The Omaha figures are now running almost as high as they were at North Platte nine years ago. The maximum count anywhere for a single day (399) has been found at Des Moines.

The season of active pollination is confined mostly to August. In the drier parts of the Dust Bowl the onset of pollination is about the same as that of Russian thistle (early July), but in the Mississippi Valley it coincides more closely with the onset of ragweed pollination. Active shedding of pollen terminates by the first week of September, after which there are only stray granules in the air.

As noted above, *Kochia scoparia* pollen grains are easily identified. They are even distinguishable from the grains of *Kochia americana* and *Kochia californica*. While the shape, as well as the number and arrange-

ment of the pore pits, is typical for granules of the order *Chenopodiales,* the size is distinctly larger and the extine in the dry state more rough and opaque than any other of the related pollens. When the grains are fully expanded, the diameter varies from 29 to 40 μ, averaging 36 μ, as compared with 25 for Russian thistle and 20 for common ragweed.

In order to appreciate the significance of the gravity slide figures for kochia pollen incidence, it is helpful to compare the volume, density, and rate of fall of kochia pollen grains with similar data for ragweed pollen grains. The relative volume is obtained by comparing the cubes of 20 and 36, respectively. This calculation shows that the kochia pollen grains have nearly six times the volume of ragweed grains. The weight is found by counting samples of a quantitative suspension of pollen

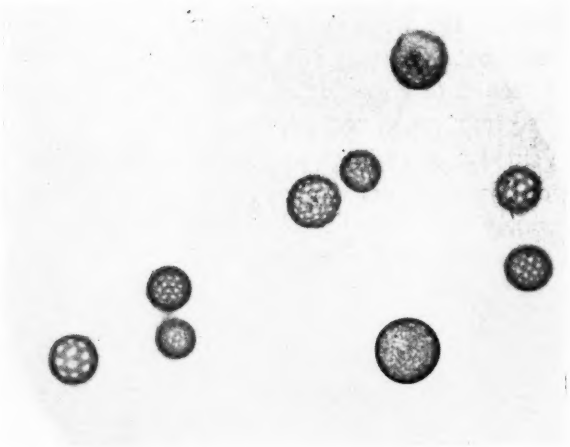


Fig. 3.—Pollens of Kochia, Russian thistle, and lamb's-quarters.

grains in lactophenol solution, using an ordinary blood counting chamber. My figures for kochia are 90,500,000 granules per gram and for ragweed, 550,000,000. Thus the mass of the former, as well as the volume, is approximately six times that of the latter. According to Cocke,[13] the rate of fall of a spherical pollen grain of 36 μ diameter is more than three times that of ragweed pollen calculated at 20 μ diameter. This makes the numerical incidence of kochia per unit volume of air considerably less than one-third of its gravity figure. But when this factor is balanced by the greater antigenic mass per grain, the resulting "contact factor" turns out to be 1.6 in favor of kochia. From this we may conclude that the gravity figures for kochia may be used for comparison with ragweed, without conversion, or if we wish to be more exact, we may multiply by 1.6. In calculating the graphic data on the accompanying map, the kochia gravity figures were given the advantage over ragweed of being multiplied by this factor.

Evaluation of the contact hazard from kochia cannot be complete without comparing it with Russian thistle, which is doubtless the most

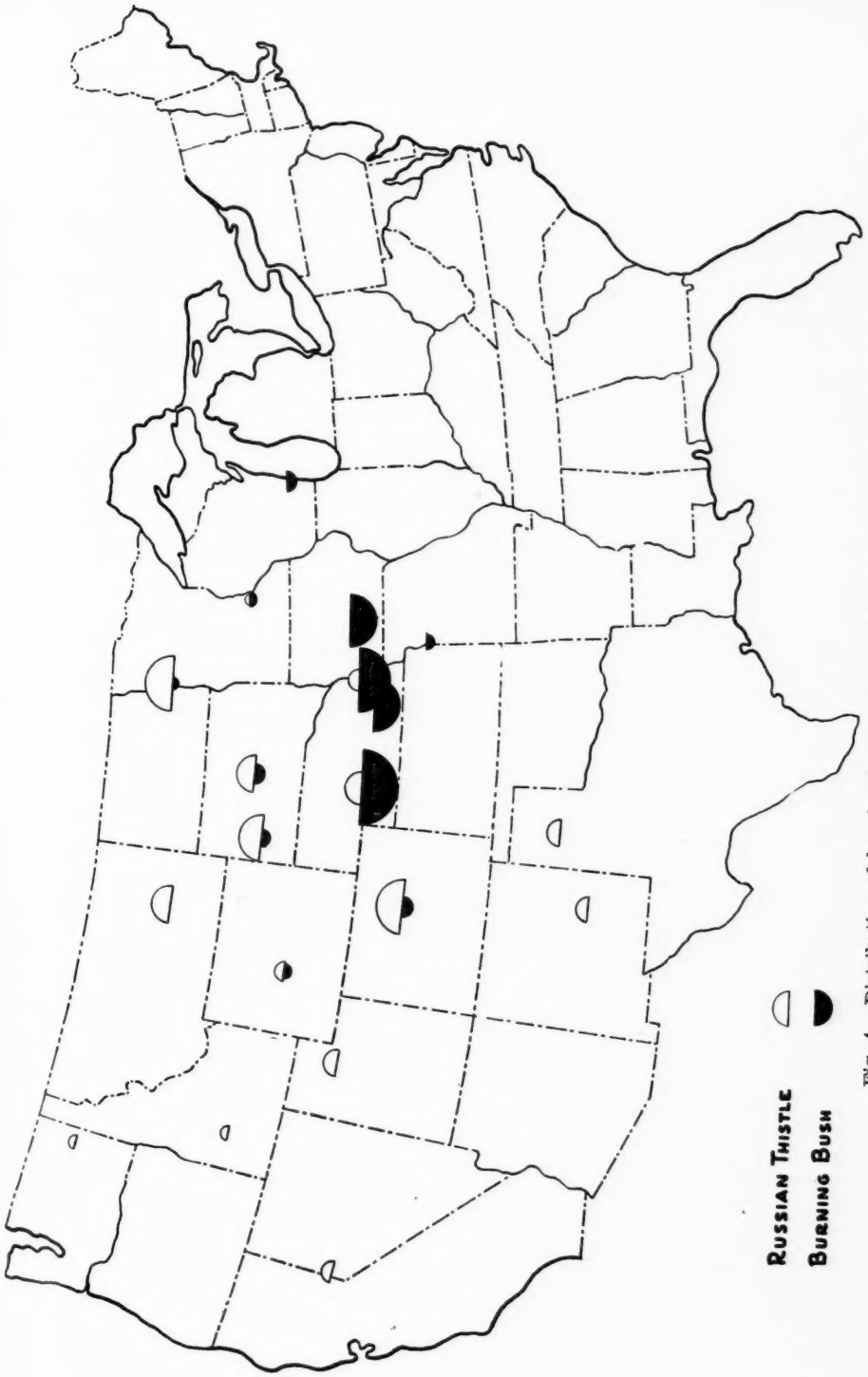


Fig. 4.—Distribution of burning bush and Russian thistle over the United States.

important member of the *Chenopodiales* order. Here we find that the volume of a kochia pollen grain is approximately three times that of a Russian thistle pollen grain. The mass ratio is slightly less—256,000,000 granules per gram. The rate of fall of Russian thistle pollen is less than half that of kochia. A calculation with the exact figures for mass and rate of fall gives a contact factor 1.7 in favor of Russian thistle. Thus, on the practical basis of probable contact of a sensitive membrane, kochia gravity figures may be compared with Russian thistle and ragweed figures as we count them on the slide without introducing any grave errors in concept. On the distribution map of kochia and Russian thistle, the totals of the latter are calculated by multiplying gravity figures by 1.7.

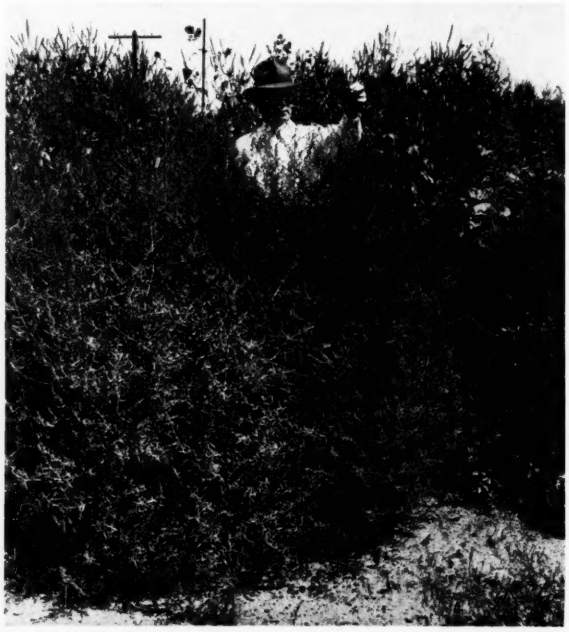


Fig. 5.—Burning bush (*Kochia scoparia*) growing in wasteland in the stockyard area of Chicago.

Now that we have found several states where the exposure to kochia pollen equals or exceeds the maximum exposure to Russian thistle pollen anywhere, it only remains to determine the relative antigenicity of the two offenders. Unfortunately in Sellers'[14] comparison of the antigenic properties of various members of the *Chenopodiales* by the method of exhaustion of passive transfer sites, he did not include kochia. MacQuiddy[15] says that the skin reactions obtained with kochia pollen are about the same size and intensity as those produced by other goosefoot pollens. My impression is that kochia pollen is somewhat less active in scratch testing than Russian thistle pollen. In order to test my impression I tabulated eighteen consecutive records of skin reactions pass-

ing over my desk on which there were positive reactions to Russian thistle. Most of these records have, of course, come from localities in which Russian thistle is an active inhalant factor. The mean of the ratings on these eighteen patients is shown in Table II. Thus, it would seem fair to conclude that kochia pollen is almost if not quite as active as Russian thistle pollen, that it contains much the same antigenic element, and that specific cases of Kochia sensitiveness in the Russian thistle area cannot be distinguished from Russian thistle cases. However, in new areas of kochia infestation where Russian thistle is present only in negligible amounts, it is entirely possible that original cases of kochia sensitiveness will develop either among ragweed victims or independent of ragweed sensitiveness.

TABLE II

SKIN REACTIONS WITH TESTS TO VARIOUS SPECIES OF CHENOPODIALES
RESULTS ON 18 PATIENTS, RATED 1+, 2+, 3+, 4+

																		AVERAGE
Russian thistle	2	2	3	4	2	4	4	4	3	4	4	3	4	3	4	3	3	3.28+
Burning bush	1	2	2	4	1	4	1	2	2	2	3	2	3	3	4	2	3	4	2.50+
Pigweed	1	1		2	0			0	1				1	4	4	0		1	1.36+
Lamb's-quarters	2			0	1			0	2	2			2	2	4	0	3	0	1.8+
Western water hemp	3			3	1	3	1			3		1			4	2		0	2.1+

My observation leads me to conclude that the seeds of burning bush have been scattered by means of hay cut from infested western fields. In Chicago and several other cities the focus of infestation is certainly in the stockyard area. I have seen plants spring up where none had ever been seen before as a result of the establishment of a horse barn and the feeding of baled hay. The source of the hay was not traced.

Whether burning bush will ever become a serious agricultural menace in the north central states and whether it will ever replace ragweed in sufficient acreage so as to diminish appreciably ragweed atmospheric contamination are questions that cannot be answered at this time. The rapidity with which the plant has spread in Chicago, Milwaukee, St. Louis, and Detroit suggests that it may soon be as common in adjacent farming districts as it has become in Colorado and Nebraska. The test of its ability to compete with other weeds where rainfall is heavier than in Nebraska is now taking place in western Iowa. It may not be safe to base our judgment on the performance of Russian thistle, which advances into the farming area during very dry seasons but is forced to recede when rainfall is heavier. If kochia should succeed in replacing as much as one-half of the acreage of ragweed in the farming area of the Mississippi Valley, the reduction of the ragweed content of the air could probably hardly be noticed by ragweed sufferers. An ap-

preciable volume of a new and unrelated air-borne allergen in that area would certainly complicate a difficult situation.

Other interesting conjectures are whether burning bush will travel on down the Mississippi from St. Louis and whether it will cross the Appalachians. For many years small colonies of the weed have been found from time to time along the Atlantic seaboard. These have been accounted for by seeds brought in with ship ballast, but these growths have been restricted and local. Certainly they have not served as centers of widespread invasions. Perhaps soil or climatic conditions on the seaboard are unfavorable or the strains brought there were not as virile as that from which the western tribe has come.

So far the acreage of weeds in the newly infested areas is far more impressive than the output of pollen. It may be that with a greater amount of moisture the plant expends its energy in the vegetative phase rather than in pollen production. It is certain, however, that it takes only a small amount of chenopod pollen to cause a great deal of suffering, as evidenced by the fact that average atmospheric concentrations of chenopod pollen in the local areas where it is active are far below ragweed concentrations in the ragweed belt.

Allergists will do well to watch this plant. It can be easily found in waste places long before its pollen will be found in the air. In areas where it has become at all common the pollen should be used in routine pollen testing.

SUMMARY

1. During recent decades, particularly the last, *Kochia scoparia* (Mexican fireweed, burning bush, summer cypress), a pernicious introduced weed of the goosefoot family, has spread rapidly in eastern Colorado, western Kansas, the Dakotas and western Iowa.

2. It has now become established in urban centers as far east as Detroit and as far south as St. Louis. Atmospheric concentration of the pollen of *Kochia scoparia* has now reached significant levels as far east as central Iowa.

3. The plant is a successful rival of Russian thistle and is already replacing ragweed locally.

4. The present hazard is that of a new, active inhalant pollen in the middle Mississippi Valley where ragweed has heretofore been dominant.

REFERENCES

1. Georgia, A. E.: A Manual of Weeds, New York, 1920, The Macmillan Co.
2. Kinch, R. C.: Nebraska Weeds, Bull. No. 101, Division of Weed Control of the Dept. of Agriculture and Inspection, Lincoln, Neb., January, 1939.
3. Standley, P. C.: North American Flora (Chenopodiales), Vol. 21, Parts 1 and 2, The New York Botanical Garden, New York, 1916, 1917.
4. Selfridge, G.: Spasmodic Vasomotor Disturbances of the Respiratory Tract, With Special Reference to Hay Fever, California State J. Med. **16**: 164, 1918.
5. Mullin, W. V.: Pollen and Hay Fever—A Regional Problem, Colorado Med. **20**: 96, 1923.

6. Waring, J. J.: Report of the Hay Fever Research Committee of the State Historical and Natural History Society of Colorado, Denver, 1926.
7. Norris, E. L.: Nebraska Weeds, Bull. No. 101, State of Nebraska Dept. of Agriculture, Lincoln, Neb., June, 1929.
8. Gates, F. C.: Weeds in Kansas, Kansas State Board of Agriculture, Topeka, June, 1941.
9. Cole, Julia: Personal communication.
10. Koessler, K. K., and Durham, O. C.: A System for an Intensive Pollen Survey, J. A. M. A. **86**: 1204, 1926.
11. Feinberg, S. M., and Durham, O. C.: Hay Fever in Chicago and Suburbs. Clinical, Field and Air Observations, Illinois M. J. **63**: 464, 1933.
12. Eyermann, C. H.: Personal communication.
13. Cocke, E. C.: Calculating Pollen Concentration of the Air, J. ALLERGY **8**: 601, 1937.
14. Sellers, E. D., and Adamson, W. B.: A Study of the Apparent Atopic Similarity of Certain Chenopodiales Pollens, J. ALLERGY **3**: 166, 1932.
15. MacQuiddy, E. L.: Personal communication.

DISCUSSION

DR. E. L. MACQUIDDY, OMAHA.—Mr. Durham's paper is of special interest to us in the Middlewest where we have considerable areas covered by kochia. It has been interesting through the years to watch the spread of this weed. There has been a distinct increase each year until now it is probably as common a weed as we have in Nebraska.

About fifteen years ago we began testing for this weed and these tests were continued over a period of approximately five years. During these years no positive reactions were found. The testing for kochia was then discontinued and not begun again until late in the season of 1940, when two reactions were found. Records on kochia in 1941 showed six reactions on seventy-five patients tested.

In 1942, our records to this date, show that we have tested seventy-seven patients and have found seventeen reactions to kochia. None of these patients reacted to kochia alone; they always reacted in association with other pollens—with Russian thistle, 8 times; giant ragweed, 5 times; Western water hemp, 4 times; lamb's-quarter, 3 times; the remainder were scattered out among eight other pollens.

How much of a factor these positive reactions to kochia play in the production of hay fever in this locality, I am unable to say; we are including kochia extract in the treatment of these seventeen patients this year. Since, however, these patients have reacted to other weeds, it is going to be difficult to draw accurate conclusions. Our plan is to continue this method for two to three years more and see if at the end of that time we will be able to say whether kochia plays a part in the production of hay fever symptoms in Nebraska.

DR. HARRY L. HUBER, CHICAGO.—In Chicago last year the newspapers called attention to the presence of this plant in the Chicago area and warned all the hay fever patients that some of their troubles might be due to this plant. It also gave all of us treating hay fever a little explanation as to why the patients did not get as good results as we hoped they would.

We started in soon after this to test our patients with the pollen of this plant, and we have found that approximately 10 per cent gave positive reactions.

Although the plant is closely related, as pointed out by Durham, to the Russian thistle, we find that not all those who are sensitive to the kochia are sensitive to Russian thistle, and vice versa. Some of them are sensitive to the Russian thistle and not to the kochia.

We have been treating patients with the Russian thistle for several years and this year have included kochia in the treatment of those patients who are sensitive or who have shown sensitiveness.

The last statement that Mr. Durham made I think is very important, that the allergist must pay attention to these things. I want to call attention to another factor and ask Mr. Durham about this. In the Chicago area we have commonly 5 grasses. There is a new one and is now more important, I believe, than orchard grass or June grass and Canada blue grass, and that is the Italian rye. As you drive out over the countryside you will find more Italian rye than you will find any other plant in Chicago. We have found that many patients are very sensitive, as the pollen of this plant is very toxic. Not all of those who are sensitive to timothy and June grass are sensitive to Italian rye, and not all of those, by any means, who are sensitive to Bermuda grass or Johnson grass are sensitive to Italian rye or to timothy.

I think in the treatment of our patients we should not limit our grass treatment to timothy extract alone, as has been advocated, as there are many patients who will not give positive reactions to some of the grass pollens and will give very definite positive reactions to others. At the same time we must keep in mind that multiple sensitizations are frequent in those who have had symptoms any length of time and that if one becomes sensitive to one he develops new sensitizations more easily and will give positive reactions to more of these pollens. Whether they are of great significance is a question.

We have found that 10 per cent of our ragweed cases are definitely sensitive to Russian thistle and to the kochia pollen, and only about 3 per cent of those sensitive to the Russian thistle were not sensitive to the kochia, and vice versa.

DR. ORVAL R. WITHERS, KANSAS CITY, MO.—I was glad to hear Mr. Durham discuss this plant. We have been testing for it in Kansas City for the past 5 years. Although I do not know the exact incidence of the reactions, I know that it is not uncommon to get reactions to kochia. I am still one of the old school who puts a little of each pollen in the treatment set. I know it is not uncommon to include kochia along with other fall pollens that we use in treatment.

Kochia is found quite extensively in Kansas City. It is especially found in vacant lots and around bridges and in areas of that type. It also grows profusely in the suburbs.

MR. DURHAM.—I think there is nothing to add except to suggest studying the atmospheric incidence of other species of kochia pollen in districts where other species are present.

Regarding Dr. Huber's comment on Italian rye grass, I had not noticed the plant being particularly abundant in Chicago. The seeds are included in commercial lawn grass mixtures, but the plants are not usually as persistent as bluegrass. The pollen is, of course, quite active, perhaps more so than some of the other grass pollens. I think it hardly likely that Italian rye grass will be able to establish itself in competition with the native quack grass and the widely distributed bluegrass and timothy.

THE QUANTITATIVE DETERMINATION OF PROTEINS IN ALLERGENIC EXTRACTS BY THE BIURET REACTION

Preliminary Report

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THIS investigation was carried out for the purpose of developing a colorimetric method for the determination of the protein nitrogen content of allergenic extracts which would eliminate the digestion and distillation procedures involved in the use of the Kjeldahl method.

That this reaction could be used as the basis of a quantitative colorimetric method for the determination of albumin and globulin in blood serum and urine has long been known.[1-9] In 1940, Robinson and Hogden[10, 11] perfected methods utilizing the biuret reaction for the determination of proteins of human, rabbit, and dog sera, applicable to spectrophotometers and visual colorimeters. Using a photoelectric colorimeter, a modification of this method was found to be suitable for the determination of protein nitrogen in allergenic extracts.

METHOD

Reagents: 3 per cent sodium hydroxide solution; 20 per cent copper sulfate solution ($CuSO_4 . 5H_2O$). A known volume (1 to 9 c.c.) of extract is placed in a 12 c.c. conical Pyrex centrifuge tube, calibrated at 10 c.c. volume. The protein is precipitated with phosphotungstic acid, after acidification with hydrochloric acid, centrifuged, and adequately washed according to the method of Cooke and Stull.[12] The washed protein precipitate is completely dissolved in 3 per cent sodium hydroxide solution by stirring and the final volume made up to 10 c.c. with the same solution.

Then 0.25 c.c. of 20 per cent copper sulfate solution is added, the tube tightly stoppered, and the mixture immediately shaken for exactly one minute, allowed to stand for thirty minutes, and centrifuged for ten minutes at 2,500 r.p.m. in an angle centrifuge.

The supernatant, approximately 10 c.c., is carefully decanted into a colorimeter tube and the reading taken. A similarly treated tube originally containing 10 c.c. of 3 per cent sodium hydroxide solution and 0.25 c.c. of 20 per cent copper sulfate solution, is used as a blank. A filter of wave length of 565 mμ is used, being available to this laboratory, although a filter of 560 mμ was used by Robinson and Hogden. In these experiments an Evelyn photoelectric colorimeter was used.

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Read at the Eighteenth Annual Meeting of the Society for the Study of Asthma and Allied Conditions, Atlantic City, N. J., May 2, 1942.

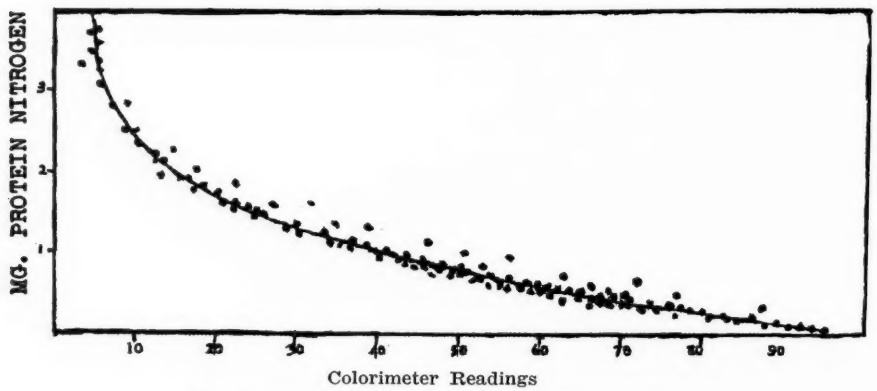

Fig. 1.—Biuret values of thirty allergen extracts.

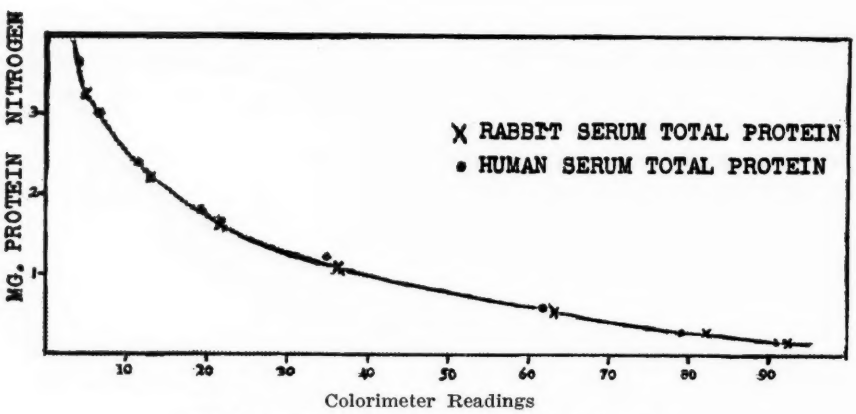

Fig. 2.—Biuret values of human and rabbit serum.

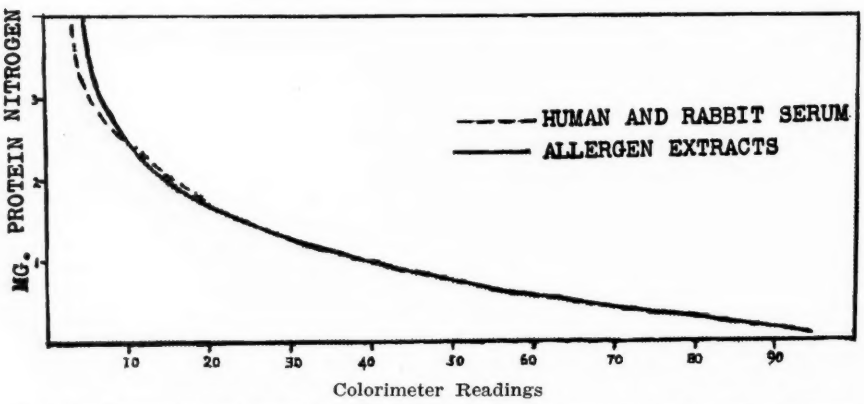

Fig. 3.—Comparison of mean biuret values of allergen extracts with those of human and rabbit serum.

The above method was adopted after the determination of the optimum time of shaking, time of color development, and time and speed of centrifugation.

The protein precipitate must be adequately washed to remove substances present in some extracts which reduce the copper in the biuret solution. The presence of such substances in extracts eliminates the possibility of running biuret determinations on the whole extract without precipitation of protein and subsequent washing.

The redissolved protein precipitate is made up to volume before the addition of the copper sulfate solution. This step is taken because the bulkiness of the copper hydroxide precipitate formed prevents the volume from being accurately measured and because immediate shaking of the mixture is imperative to obtain thorough mixing.

Since it was not known how the biuret values of the different types of protein found in a variety of extracts would coincide, a series of determinations were made, in the above manner, on thirty different colorless or nearly colorless extracts representing various foods and inhalants. At least three different quantities of each extract were used. These readings were plotted against values obtained by the Kjeldahl method on the redissolved protein precipitates of each extract. These then were reduced to a mean value, and this was plotted as a standard curve (Fig. 1). In the Kjeldahl determinations, boric acid was used in the distillation of the ammonia.[13] The most desirable range of concentration of protein nitrogen per sample is from 0.10 to 2.00 mg.

Biuret values were obtained on normal human and rabbit sera total protein (Fig. 2). The mean values for both sera were identical as was found by Robinson and Hogden. The curve derived from the thirty different allergenic extracts was compared with that derived from human and rabbit sera, as determined by the method used in these experiments. It is interesting that although the two curves represent different types of protein, they practically coincide (Fig. 3).

RESULTS

Thus far, determinations by this biuret method on sixty-two colorless extracts checked repeatedly with those obtained by the Kjeldahl method within an error of ± 0.05 mg. of nitrogen per cubic centimeter (Table I).

Checks were never obtained with extracts of glue, green pea, and horse dander, even upon analysis of four different extracts of each. The Kjeldahl values far exceeded the biuret values.

The reason for discrepancies of values on some extracts may be the fact that the precipitative reagent, phosphotungstic acid, used in this experiment is not specific for pure proteins alone, but it also is capable of precipitating basic amino acids as well as proteoses, peptones, and polypeptides. This fact, on the one hand, may lead to unreliable Kjeldahl values and, on the other, give equally unreliable biuret values since this reaction is characteristic of all proteins, peptones, and certain

TABLE I

COMPARISON OF BIURET AND KJELDAHL VALUES OF COLORLESS EXTRACTS (PHOSPHOTUNGSTIC ACID PRECIPITABLE NITROGEN)

EXTRACT	BIURET (MG. N/C.C.)	KJELDAHL (MG. N/C.C.)	EXTRACT	BIURET (MG. N/C.C.)	KJELDAHL (MG. N/C.C.)
Almond	0.61	0.62	Lamb	0.72	0.73
Apple	0.01	0.01	Lentil	0.06	0.08
Apricot	0.03	0.03	Lettuce	0.004	0.002
Artichoke	0.04	0.07	Lima bean	0.23	0.25
Asparagus	0.02	0.04	Milk, whey	0.76	0.74
Banana	0.03	0.04	Mustard, green	0.01	0.01
Barley	0.15	0.17	Navy bean	0.50	0.54
Beef	0.56	0.57	Nuts, mixed	0.76	0.76
Brazilnut	1.00	1.00	Oatmeal	0.03	0.02
Broccoli	0.03	0.02	Oats, whole	0.14	0.13
Cabbage	0.04	0.004	Olive	0.008	0.008
Cantaloupe	0.02	0.03	Onion	0.01	0.03
Carrot	0.01	0.01	Orris root	0.23	0.24
Casein	0.28	0.28	Parsley	0.01	0.01
Cat Ep.	0.04	0.07	Pea	0.04	0.06
Cauliflower	0.04	0.04	Peach	0.01	0.01
Celery	0.01	0.005	Peanuts	0.30	0.30
Chicken	0.17	0.18	Plum	0.001	0.001
Coconut	0.08	0.09	Pork	0.63	0.63
Cornmeal	0.14	0.12	Potato, sweet	0.15	0.14
Corn, whole	0.24	0.25	Potato, white	0.15	0.17
Cottonseed	0.15	0.14	Rabbit Ep.	0.07	0.12
Cucumber	0.02	0.04	Radish	0.002	0.003
Eggplant	0.004	0.004	Rice	0.01	0.01
Egg white	0.53	0.53	Rye flour	0.16	0.18
Feathers	0.03	0.04	Soy bean	0.49	0.51
Fish	0.88	0.89	Squash	0.01	0.02
Flaxseed	0.40	0.40	String bean	0.23	0.26
Garlic	0.18	0.20	Turnip	0.03	0.04
Glue	1.58	1.98	Watermelon	0.04	0.05
Green pea	0.39	0.47	White flour	0.12	0.11
Honey	0.02	0.01	Whole wheat	0.21	0.19
Horse dander	0.18	0.27	Yeast	0.01	0.01
Horse serum	0.51	0.51			
Human serum	0.42	0.42			
Kapok	0.05	0.05			

nonprotein materials which have two amino groups attached to different carbon atoms. Preliminary investigation using other precipitants indicates that this may be the case.

At present, this method can be applied only to colorless or nearly colorless extracts. A modification is being perfected for its application to dark-colored extracts, including pollen.

SUMMARY

This preliminary report is presented to illustrate a possible colorimetric method for the quantitative estimation of protein nitrogen of extracts of allergens which would have several advantages over the Kjeldahl method and which could be applied as a possible short-cut in the Cooke and Stull method of standardization.

I am indebted to Dr. Ethel Ronzoni Bishop, of the Department of Biochemistry, for helpful suggestions and criticism.

REFERENCES

1. Autenreith, W., and Mink, F.: Ueber kolorimetrische Bestimmungsmethoden: Die quantitative Bestimmung von Harneiweiss, München. med. Wchnschr. **62**: 1417, 1915.
2. Autenreith, W.: Ueber kolorimetrische Bestimmungsmethoden: Die Bestimmung von Serumalbumin und Globulin im Harn, in der Aszitesflussigkeit und im Blutserum, München. med. Wchnschr. **64**: 241, 1917.
3. Hiller, A.: Determination of Albumin and Globulin in Urine, Proc. Soc. Exper. Biol. & Med. **24**: 385, 1927.
4. Hiller, A., McIntosh, J. F., and van Slyke, D. D.: The Excretion of Albumin and Globulin in Nephritis, J. Clin. Investigation **4**: 235, 1927.
5. Fine, J.: The Biuret Method of Estimating Albumin and Globulin in Serum and Urine, Biochem. J. **29**: 799, 1935.
6. Lieben, F., and Jesserer, H.: Studien zur Biuretreaktion des Proteine, Biochem. Ztschr. **285**: 36, 1936.
7. Sizer, I. W.: The Biuret and Ninhydrin Tests for Proteins as Measured With Hardy's Spectrophotometer, Proc. Soc. Exper. Biol. and Med. **37**: 107, 1937.
8. Kingsley, G. R.: The Determination of Serum Total Protein, Albumin and Globulin by the Biuret Reaction, J. Biol. Chem. **131**: 197, 1939.
9. Jesserer, H.: II Mitteilung: Ueber Metallverbindungen des Biurets, Biochem. Ztschr. **287**: 71, 1936.
10. Robinson, H. W., and Hogden, C. G.: The Biuret Reaction in the Determination of Serum Proteins. I. A Study of the Conditions Necessary for the Production of Stable Color Which Bears a Quantitative Relationship to the Protein Concentration, J. Biol. Chem. **135**: 707, 1940.
11. Robinson, H. W., and Hogden, C. G.: The Biuret Reaction in the Determination of Serum Proteins. II. Measurements Made by a Duboscq Colorimeter Compared With Values Obtained by the Kjeldahl Procedure, J. Biol. Chem. **135**: 727, 1940.
12. Cooke, R. A., and Stull, A.: The Preparation and Standardization of Pollen Extracts for the Treatment of Hay Fever, J. ALLERGY **4**: 87, 1933.
13. Sobel, A. E., Yuska, H., and Cohen, J.: A Convenient Method of Determining Small Amounts of Ammonia and Other Bases by the Use of Boric Acid, J. Biol. Chem. **118**: 442, 1937.

DISCUSSION

DR. ROBERT A. COOKE.—I was very glad to be asked to open discussion on this paper because it indicates a definite broadening of the interest in chemical studies of allergens with which we deal so much in our work.

There has, I think, up to the present time been too much hit-or-miss manufacture of extracts with no attempt to carefully analyze, study, and determine just exactly what we were using. As long as we could get a positive reaction with an extract, it was allowed to go at that.

The point is whether or not standardization is of importance. My own feeling is that it is of great importance, and yet, when we ask ourselves the question, "What do we mean by standardizing?" of course we would like to feel that it was not only a chemical but a biologic standard as well.

The difficulties that arise in standardizing all of our extracts today are largely dependent upon the fact that the chemical standardization does not necessarily agree with the biologic, although with certain precautions and with care of the extracts there is no question but that the chemical standardization does approach the biologic. The reason, of course, is that in these extracts there is an autolytic process going on, one that does not completely denaturize the protein but alters it or at least modifies it as far as its biologic activity is concerned. It was on that account that some years ago we attempted to stabilize the unstable protein in these solutions by various means, and yet, frankly, we were not entirely successful.

However, there is no doubt but that any method that will facilitate the protein estimation is going to be a great advantage because there is no question but that the Kjeldahl method is, while accurate, cumbersome and time-consuming.

The method that has been presented to us for a simpler and easier technique is one I am sure that we will all welcome, if, as, and when it is perfected in some of the details that Miss Johnson still has under consideration.

For example, you notice that she emphasizes the point that these extracts must be colorless and you also notice that she has not yet presented any data with regard to the question of the pollen extracts which are particularly important because they are so largely used for therapeutic purposes. However, these are, I am sure, the problems that Miss Johnson has in mind for future attack and for future presentation. I really feel that this is an important and interesting step, and I think that we should all be very pleased that we have heard so nicely presented this new approach which will make standardization from the chemical angle much easier and much simpler for all of us.

A PHARMACOLOGIC STUDY OF EPINEPHRINE SUSPENSIONS IN OIL

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ABOUT four years have elapsed since Keeney[1] first described the use of a suspension of epinephrine in oil for the treatment of asthmatic and other allergic conditions. Subsequent publications[2-4] by Keeney as well as by other investigators[5-7] have indicated that in principle there is agreement that this form of medication represents a valuable addition to the therapy of these conditions because of the prolonged action, resulting in a relief of symptoms for several hours.

Within the last few years a number of reports of undesirable side-effects as a result of the administration of epinephrine in oil suspensions have appeared in the literature.[8-11] These side-effects are of two types: a local one due to hypersensitivity to the oil or also occasionally to epinephrine,[12] and the other due to systemic reactions to the epinephrine. Among the latter group, a differentiation can be made between a genuine hypersensitivity to epinephrine, independent of its form of administration, and a too rapid rate of absorption. Suggestions have been made to reduce the danger of the latter possibility by the use of small-gauge needles[13] and by keeping the dose as low as possible.

It has been pointed out by Sterling[14] that therapeutically useful doses of epinephrine hydrochloride (0.5 to 1.0 mg.) can cause dangerous symptoms and may even result in death. A review of the literature shows that doses up to 1.5 mg. of epinephrine in the form of a suspension in oil have been given. It seems that the majority of side-reactions have followed the administration of such relatively high doses, since even the greatest care will not always be able to prevent an undesirably rapid absorption. Nausea, pallor, a feeling of oppression and fear, tachycardia, and hypertension have been reported as sequences of such accidents.

A search of the literature of recent years reveals only scant experimental data on epinephrine suspensions. Kabak and Freedman[15] found that the injection of such suspensions prolongs life and sustains blood pressure in experimental shock in animals.

Keeney,[2] in one of his first publications, compared the blood sugar response of patients to epinephrine in oil suspension and aqueous solution. He found that the former type resulted in a less pronounced but more prolonged effect upon the blood sugar. A similar difference appears to exist in the cardiovascular response.

EXPERIMENTS

Our intention was to gather information regarding certain toxicologic and pharmacologic properties of epinephrine in oil suspensions as com-

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pared with aqueous solutions of the hydrochloride. To determine the stability and potency of the epinephrine in the oil suspensions, the epinephrine was extracted by repeatedly shaking the oil suspension with aqueous hydrochloric acid solution in a separatory funnel and the resulting aqueous solution assayed by the determination of its pressor effect in dogs. It was shown by this method that the suspended epinephrine can be completely recovered, and also that the stability of the suspensions is satisfactory over a period of many months.

The activity of the epinephrine in oil suspensions was further investigated by determining the toxicity as compared with epinephrine in aqueous solutions. Rats were used for these experiments. The injections were made intramuscularly in the hind legs with a 26-gauge needle, care being taken to avoid leaking out of the injected material. An 0.2 per cent suspension of epinephrine in peanut oil, as it is prepared commercially, was used and compared with an aqueous solution of epinephrine hydrochloride 1:1,000, as described in the U.S.P., without the addition of chlorbutanol or sodium bisulfite. The doses refer always to epinephrine base. The results are given in Table I.

TABLE I

TOXICITY OF EPINEPHRINE BY INTRAMUSCULAR INJECTION IN RATS

	DOSE (MG. PER KG.)	NUMBER USED	NUMBER FATAL	% FATAL
Epinephrine suspension in peanut oil 2 mg. per c.c.	3	18	9	50.0
	4	52	38	73.1
	6	36	32	88.9
Epinephrine HCl aqueous sol. 1 mg. per c.c.	2	15	0	0.0
	3	23	13	56.6
	4	39	32	82.1
	6	18	18	100.0

It is evident from this table that the toxicity of epinephrine in the oil suspension is practically the same as that of epinephrine in aqueous solution. Toxic symptoms after the injection of both forms were of an identical nature. The animals became cold and restlessness and convulsions appeared, followed by dyspnea, prostration, and, frequently, pulmonary edema. The course of the poisoning varied greatly insofar as the symptoms sometimes began within five to ten minutes after the administration, and deaths occurred anywhere from thirty minutes to twenty-four hours after the injection. While the onset of these symptoms appeared to be somewhat more delayed with the epinephrine in oil suspension as compared with the aqueous solution, there was a good number of instances in which the suspension form also caused convulsions and death within a short time, thus indicating the difficulty of controlling the absorption. Clinically, Bacon and collaborators[16] have shown that epinephrine suspensions in oil do not vary significantly in speed of onset or degree of effect as compared to equivalent doses of epinephrine in aqueous solution.

During the course of these experiments we had on a few occasions used commercial epinephrine hydrochloride solutions instead of freshly prepared ones. These commercial solutions were carefully bioassayed and found to be of the required potency. The only difference in these preparations was the presence of chlorbutanol and sodium bisulfite as preservative agents; but such solutions proved to be several times more toxic in rats, and we found that the small amount of sodium bisulfite is responsible for this effect. We have studied this phenomenon more closely and have reported upon it briefly.[17] A more detailed publication will soon follow. We mention this fact here since probably most of the clinical comparative studies have been made using such commercial preparations, and it is easily possible that their more powerful but briefer action may be influenced to a certain degree by the presence of sodium bisulfite.

A number of experiments designed to prove a longer action of the epinephrine in oil suspensions as compared with aqueous solutions gave inconclusive data. We followed the blood sugar curve after the injection of identical amounts of epinephrine in the two forms of administration in dogs and rabbits, and while there was some indication of a more prolonged effect with the suspension, the results were not impressive. Similarly, we were unable to find a much longer action of the oil suspension by measuring the dilating effect of the epinephrine upon the pupil in rabbits after the removal of the superior cervical ganglia. Both forms of administration resulted in a similar degree of dilation of the pupil, persisting for several hours.

It is well known that subcutaneous or intramuscular injections of epinephrine are not followed by a rise of blood pressure in animals, while in human beings such effect is observed quite regularly. It may be, therefore, that our inability to observe differences in the bood sugar and the effect upon the pupil may have to do with the species used, since, as mentioned above, Keeney[2] was able to demonstrate a more prolonged action of the suspension upon the blood sugar and cardiovascular response in human beings.

It occurred to us that the size of the particle may also influence the speed and degree of action. The sizes of particles of commercial epinephrine in oil suspension in one sample showed an average of 19 μ, with a maximum of 34.2 μ. An identical suspension was then prepared with the epinephrine ground up to a finer size, the average particle being 3.8 μ and the largest one 6.65 μ. Acute toxicity experiments in rats were conducted in the same manner as described above. While the bioassay made after shaking out of the epinephrine from the oil with hydrochloric acid water showed both solutions to contain the same amount of epinephrine activity, the toxicity experiments proved a difference in their biologic effect. The epinephrine suspension made up of the smaller particles did not give consistent toxicity data, frequently having a considerably lower toxicity. Furthermore, the potency, measured by this

method, decreased rapidly when these suspensions were stored for a few weeks. These results are not easily explainable but indicate the incompleteness of information which can be derived from assaying epinephrine in oil suspension merely by removing it from the oily phase.

Keeney[2] has reviewed the scant data which are available upon the fate of oil suspensions in the body. It must be assumed that the suspended material is resorbed much faster than the oil itself, which remains at the site of injection for a considerable period of time. It may be possible that the very small particle sizes are tightly surrounded by an oil film and thus more protected against the action of the tissue fluids. If once a larger particle is broken up, the tissue fluid has access to a relatively larger surface of material which is not oil-coated. Whether or not this explanation is a correct one cannot be stated. Furthermore, it was found that the suspension of the very fine particles was not as stable over a prolonged period of time as the larger particle sizes.

SUMMARY

1. It has been shown that the toxicity of epinephrine in oil suspension by intramuscular injection in rats is about equal to that of a freshly prepared epinephrine hydrochloride aqueous solution. Ordinary commercial epinephrine solutions containing sodium bisulfite have a considerably higher toxicity.

2. While the onset of toxic symptoms and the occurrence of deaths with the oil suspension were, as a rule, delayed as compared with aqueous solutions, there was still a significant number of instances in which symptoms of poisoning and deaths occurred as quickly as with the aqueous solutions, thus indicating difficulty in controlling the absorption of suspensions of epinephrine.

3. We were unable to demonstrate a more prolonged action of the oil suspension by following the blood sugar curve in dogs and rabbits or the pupillary dilation in rabbits.

4. It has been shown that finer particle sizes result in a less toxic and probably less potent preparation, which is also less stable than one made up of somewhat coarser particles.

REFERENCES

1. Keeney, E. L.: A Slowly Absorbed Epinephrine Preparation, Bull. Johns Hopkins Hosp. **62**: 227, 1938.
2. Keeney, E. L., Pierce, J. A., and Gay, L. N.: Epinephrine in Oil, a New Slowly Absorbed Epinephrine Preparation, Arch. Int. Med. **63**: 119, 1939.
3. Keeney, E. L.: Epinephrine in Oil, Its Effect in the Symptomatic Treatment of Hay Fever, J. ALLERGY **10**: 590, 1939.
4. Keeney, E. L.: Epinephrine in Oil, Its Effectiveness in the Symptomatic Treatment of Bronchial Asthma, Am. J. M. Sc. **198**: 815, 1939.
5. Murphy, J. A., and Jones, C. A.: Slow Epinephrine in the Treatment of Chronic Asthma, J. ALLERGY **10**: 215, 1939.
6. Thiberge, N. F.: The Use of Adrenalin in Oil and Adrenalin in Gelatin for the Relief of Allergy, M. Rec. **152**: 181, 1940.
7. Kalmon, M.: A Clinical Comparison of Epinephrine in Oil and Epinephrine in Gelatin, J. ALLERGY **12**: 55, 1940.

8. Maietta, A. L.: Systemic and Local Reactions to Epinephrine in Oil, New England J. Med. **222**: 715, 1940.
9. Cohn, J.: Unusual Reactions to Slow Epinephrine, J. ALLERGY **10**: 459, 1939.
10. Dorwart, F. G.: "Slow Epinephrine"—An Experience, J. A. M. A. **114**: 647, 1940.
11. Insley, S. W., and Segaloff, A.: Systemic Reaction to Slow Epinephrine: Correspondence, J. A. M. A. **114**: 1826, 1940.
12. Cohen, A. E., and Waterstone, M. W.: Epinephrine Hypersensitivity: Two Cases, J. ALLERGY **11**: 393, 1940.
13. Keeney, E. L.: Letter to Editor, J. A. M. A. **114**: 1098, 1940.
14. Sterling, A.: Dangers Attending Clinical Use of Epinephrine in Bronchial Asthma, M. Clin. North America **24**: 1857, 1940.
15. Kabat, A., and Freedman, A. M.: Effect of Slowly Absorbed Epinephrine in Experimental Shock, Proc. Soc. Exper. Biol. & Med. **46**: 385, 1941.
16. Bacon, L. C., Stickler, M., and Lamson, R. W.: The Speed of Action of So-Called "Slow Epinephrine" (in Oil), J. ALLERGY **13**: 48, 1941.
17. Richards, R. K.: Potentiation of Epinephrine Toxicity by Sodium Bisulfite, Federation Proceedings (Am. Pharm. Soc.), Vol. I, Part II, p. 71, 1942.

THE USE OF VITAMIN B COMPLEX IN THE TREATMENT OF INFANTILE ECZEMA

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THE term "infantile eczema" has been employed in the past to embrace a heterogeneous collection of inflammatory dermatoses of the infant and young child which are unrelated etiologically and dissimilar histologically. From this collection, from time to time, some newly assembled group of symptoms is being removed as a distinct clinical entity.

In recent years much attention has been centered upon the relation of vitamin deficiencies to the dermatoses. Gyorgy, Sullivan and Karsner[1] produced symptoms of seborrheic dermatitis in rats by feeding them raw egg white in an otherwise well-balanced diet. They were able to bring about a cure by the administration of biotin. Rats maintained on a diet deficient in pyridoxine[2] have been shown to develop "rat acrodynia," which can be cured by the administration of the filtrate factor in addition to pyridoxine.

Karunakaran and Nair[3] reported ninety-two infants with scrotal eczema, often associated with angular stomatitis, glossitis, and hyperkeratosis, which were successfully treated with vitamin A and the B_2 complex.

Pehl[4] treated eight babies with infantile seborrhea with vitamin B_6. In six the results were nil and in the other two the results were doubtful. Sebrell and Butler[5] produced skin lesions in women maintained on a diet low in riboflavin. The lesions began as a pallor of the mucosa of the lips at the angles of the mouth which were followed by maceration and superficial transverse fissures. There was also a fine, scaly, slightly greasy desquamation on a mildly erythematous base in the nasolabial folds, on the alae nasi, in the vestibule of the nose, and on the ears. Kruse, Sydenstricker, Sebrell and Cleckley[6] have reported, in addition, a keratitis associated with these signs of riboflavin deficiency. These lesions responded to the administration of synthetic riboflavin.

Kristensen and Vendel[7] reported twenty unselected infants with eczema whom they treated with a Danish brand of vitamin B complex (Bevital Leo). They reported that itching disappeared and healing began almost immediately, and in the acute cases a complete cure was obtained in a week to ten days. In the chronic cases they reported definite improvement within the first two weeks of treatment. While they advance no theories as to the responsible component, they

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state definitely that B_1 (thiamine) is of no value in the treatment of the disease.

In view of the satisfactory results reported by these workers, twenty unselected cases of childhood eczema were treated in this clinic with vitamin B complex.* Children under the age of 1 year were given 2 c.c. three times a day, and those over 1 year of age were given 4 c.c. three times a day. These children ranged in age from 6 months to 12 years, averaging 4.2 years. The duration of the eczema ranged from 5 months to 11.5 years, averaging 3.3 years. The period of treatment ranged from two weeks to ten months. Table I indicates the details of the treatment, the extent and duration of the lesions, and the results obtained.

TABLE I

CASE	AGE	DURATION	DISTRIBUTION	PERIOD OF TREATMENT	RESULT
J. B.	15 mo.	5 mo.	Face and extremities	3 wk.	Improved
J. C.	2½ yr.	2 yr.	Face and extremities	2½ mo.	Improved
A. H.	9 mo.	7 mo.	Face, trunk, and extremities	9 mo.	Improved
M. J.	10 yr.	9 yr.	Arms, face, and neck	6 wk.	Improved
B. K.	2½ yr.	2 yr.	Face and extremities	2 wk.	Improved
S. P.	1 yr.	4 mo.	Face	13 mo.	Cured
M. S.	10 yr.	2 yr.	Arms and legs	1 mo.	Improved
N. W.	3½ yr.	3 yr.	Hands and legs	3 wk.	Improved
F. A.	9½ yr.	9 yr.	Generalized, severe	3 wk.	No improvement
W. B.	21 mo.	20 mo.	Generalized	4 mo.	No improvement
J. C.	6 mo.	5 mo.	Face and legs	11 mo.	No improvement
V. H.	12 yr.	11½ yr.	Arms and legs	5 mo.	Improved
D. A.	4½ yr.	4 yr.	Face	7 mo.	No improvement
G. E.	15 mo.	13 mo.	Generalized	10 mo.	Improved
S. F.	4 yr.	3½ yr.	Arms and legs	2 wk.	No improvement
B. P.	10½ yr.	10 yr.	Face and legs	8 mo.	No improvement
R. S.	5 yr.	4½ yr.	Arms and legs	10 mo.	No improvement
J. S.	18 mo.	8 mo.	Face and extremities	11 mo.	Cured
T. K.	9 mo.	8 mo.	Face and extremities	1 mo.	Improvement
A. R.	9 mo.	6 mo.	Face, neck, and extremities	1 mo.	Slight improvement

Many of these patients were known to be sensitive to certain articles of diet, but no change in diet was made during this study.

*The preparations used in this study were Elixir of Betalin Complex (Lilly) and Elixir Beta-Concemin (Merrell).

Betalin Complex (fluid ounce):

B_1	1,000 International Units
Riboflavin	480 Sherman Units
Nicotinic acid	12 mg.
B_6 and other factors derived from 80 Gm. fresh liver.	

Beta-Concemin (fluid ounce):

B_1	2,664 International Units
B_2	2,664 gammas
Nicotinic acid amide	28 mg.
Pantothenic acid	600 gammas
B_6, factor W, and other components of vitamin B complex derived from liver.	

Of the twenty infants treated in this study, all but two of them showed an immediate improvement, indicated by a decrease in itching and a tendency toward healing. In the final analysis, however, it was found that treatment in two infants resulted in complete healing, eleven showed varying degrees of improvement, and the remaining seven showed no change.

SUMMARY

1. From a small series of twenty cases it would, of course, be impossible to draw definite conclusions.

2. While we were unable to obtain the uniformly excellent results obtained by other workers, we feel that vitamin B complex in some cases is a valuable adjunct in the treatment of eczema.

REFERENCES

1. Gyorgy, P., Sullivan, M., and Karsner, H. T.: Nutritional Dermatoses in Rats, Proc. Soc. Exper. Biol. & Med. **37**: 313, 1937.
2. Gyorgy, P.: Dietary Treatment of Scaly Desquamative Dermatoses of the Seborrheic Type, Arch. Dermat. & Syph. **43**: 230, 1941.
3. Karunakaran, C. O., and Nair, P. K.: The Treatment of Scrotal Eczema, Stomatitis and Allied Conditions Caused by Vitamin Deficiency, Indian J. M. Research **28**: 371, 1940.
4. Pehl, W.: Clinical Trial of Vitamin B_6 in Cases of Infantile Seborrhea, Ztschr. f. Kinderh. **61**: 613, 1940.
5. Sebrell, W. H., and Butler, R. E.: Riboflavin Deficiency in Man, Pub. Health Rep. **53**: 2282, 1938.
6. Kruse, H. D., Sydenstricker, V. P., Sebrell, W. H., and Cleckley, H. M.: Ocular Manifestations of Ariboflavinosis, Pub. Health Rep. **55**: 157, 1940.
7. Kristensen, K. P., and Vendel, S. N.: Treatment of Eczema With Vitamin B Complex, Lancet **1**: 170, 1940.

Society Proceedings

AMERICAN ASSOCIATION FOR THE STUDY OF ALLERGY

ANNUAL MEETING, ATLANTIC CITY, N. J., JUNE, 1942

Some Pathologic Aspects of Allergy. Paul R. Cannon,* M.D., Chicago, Ill. (Abstract.)

The characteristics of local allergic reactions usually recognized in human disease may be enumerated in part as follows: (1) an intensified inflammatory response associated with edema, (2) the deposition of fibrin, (3) granulocytic infiltration, (4) "fibrinoid" or other types of necrosis, (5) hemorrhage, (6) eosinophilia, (7) mononuclear and fibroblastic accumulations with accelerated healing, and (8) an increased tendency to localization of the lesion. Not all features are present in any one lesion, depending upon the nature and the concentration of the allergic agent, the degree of sensitization of the tissues, and the age of the lesion when examined.

The study of allergic lesions in human necropsy material is hampered by the fact that so frequently one must deal with end stages; consequently, objective features are obscured by other interfering factors such as age, recurrences of complicating infectious processes, and phenomena of tissue repair. Furthermore, uncertainty about the age of the lesions under observation and the varying degrees of sensitization make interpretation difficult.

It is apparent, therefore, that a sequential study of "model lesions" whereby both allergenic and allergic conditions can be more adequately controlled with study of the tissues after good fixation and staining should help to elucidate some of the problems concerned. Such lesions can be induced by protein sensitizations, by bacterial infections, and by parasitic infestations.

The questions foremost in mind are: (1) What is the essential mechanism of allergic inflammation? (2) What part does allergic inflammation play as a protective mechanism? and (3) Is allergic inflammation a "disease" which should, if possible, be eliminated?

Accumulated evidence is now abundant, but interpretation of its significance is still uncertain. Nevertheless, the following tentative conclusions seem to be warranted at the present time.

The lesion of allergic (anaphylactic) inflammation represents the tissue response which eventuates in an enhanced localizing capacity for the allergenic substance or agent. This localizing capacity varies directly with the degree of sensitization (or resistance) of the host:

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with the highest degrees of resistance, as when manifested against a living antigen, the latter may be encompasssed so rapidly that practically no growth occurs and the inflammation is consequently minimal. The effects depend, obviously, upon the quantity of antigenic material reacted to by the sensitized tissues. For example, allergic inflammation in the lung directed at one tubercle bacillus would be associated presumably with an inconsequential lesion, whereas the same tissues, when reacting against large numbers of such bacilli and their accompanying tuberculinic proteins, would cause massive inflammation and necrosis and the pathologic findings of tuberculous bronchopneumonia. And yet allergy could be the same in both situations, beneficent in the former instance and "harmful" in the latter.

Accumulated evidence indicates that the allergic lesion results from the union in the tissues of antigen and antibody. In some instances the antibodies are agglutinins, in others, precipitins or reagins. It may be, however, that all of these are only names to describe a process which is fundamentally similar if not identical in all instances. The effects may manifest themselves either intracellularly or extracellularly or in both respects, and the lesions may be visible or invisible, depending upon quantitative interrelationships. The inflammatory reaction is, therefore, essentially secondary, an intensified reaction to injury, which tends to augment the localizing potentiality of the antigen-antibody combination.

The local allergic lesions, therefore, may be looked upon, as Opie has so well expressed it, as a mechanism whereby "vital organs are protected at the expense of local injury." At times the local allergic lesions may, paradoxically, be the cause of death, as in tuberculous meningitis, edema of the larynx, laryngitis, bronchopneumonia, etc. It would seem, however, looked at from the "long view," that the local allergic lesions, disastrous and discomforting as they frequently are to the few, may, for the many, represent a beneficent protective mechanism which occasionally operates excessively to cause harm but more often conserves against greater generalized harm.

Selected Abstracts

Asthma and Hay Fever

UNDER THE DIRECTION OF SAMUEL FEINBERG, M.D., AND
THEODORE B. BERNSTEIN, M.D., CHICAGO

The Incidence of Atmospheric Mold Spores in Relation to Climatic Conditions in Milwaukee, 1935-1941. Randolph, T. G., and Squier, T. L.: Wisconsin M. J. **41**: 987, 1942.

A study of alternaria spores on slides which the authors exposed in Milwaukee during the years 1935 to 1941 inclusive is recorded on tables and graphs. They found that these spores seldom reached significant counts until the middle of July. During the latter half of July and the first half of August, the alternaria counts are heavy. It appears that in the latter part of August, there is a definite reduction in the curve of incidence. The peak occurs in the early part of September, and during the latter part of September there is a marked down trend of the curve. A secondary peak is also present the first part of October. The authors found that strong winds and south and southwest winds increased the counts, while rain caused an increase several days later.

Plate studies made in 1939-1940 inclusive showed maximum hormodendrum counts occurring early in August with significant secondary peaks in June and October.

Eosinophilia in Fatal Asthma: Studies of Bone Marrow and Myocardium. Chafee, F. H., Ross, J. R., and Gunn, E. M.: Ann. Int. Med. **17**: 45, 1942.

The authors describe the autopsy findings in a case of status asthmaticus in which a large preponderance of eosinophilic myelocytes, metamyelocytes, and leucocytes were demonstrated in the bone marrow. An acute diffuse myocarditis with marked infiltration of eosinophilic leucocytes was also present.

Prevention of Death in Status Asthmaticus. Bases, L., and Kurtin, A.: Arch. Otolaryng. **36**: 79, 1942.

Eight cases in which death was due to status asthmaticus are presented by Bases and Kurtin. The post-mortem findings in these eight cases and in those in the literature appear to indicate that the most im-

portant factor responsible for the fatal outcome in status asthmaticus is obstruction of the tracheobronchial airway by viscid secretion from the mucous glands of the bronchi. The removal of such material by bronchoscopic aspiration may be lifesaving and is regarded as a necessary measure in serious instances of status asthmaticus.

Bronchial Antispasmodic Actions of Theophylline Derivatives (Theophylline Ethylenediamine and Theophylline With Sodium Acetate), Including Effects of Continued Administration. Ludena, F. P.: Jour. Pharmacol. & Exper. Therap. **75**: 316, 1942.

The bronchial antispasmodic action of aminophyllin and theocin was studied on isolated lungs of guinea pigs and intact lungs of dogs. Both drugs acted as bronchodilators and relieved the bronchospasms of histamine, pilocarpine, barium, and anaphylactic shock. Epinephrine was at least one thousand times more effective in this action, dose for dose. Consecutive daily intravenous injections of aminophyllin for a period of a month, in doses comparable to those used clinically, did not produce any noticeable pathologic changes.

Allergic States in Selectees. Hyde, R. W.: New England J. Med. **227**: 241, 1942.

Among 45,000 registrants for military service examined at the Boston Recruiting and Induction Station, 220, or 0.5 per cent, were disqualified for general military service because of severe allergic states, chiefly chronic asthma. The author is of the opinion that even mild asthma should be a cause for disqualification for general military service because of the likelihood of frequent illness, serious sequelae, and increased risks of compensation. An adequate diagnosis could be made only in 60 per cent of the men from routine examination. It was decided that certain other findings were of value in substantiating a diagnosis. Among these were the presence of nasal sinusitis or nasal polypi, dorsal kyphosis, or an increased anteroposterior diameter of the chest, positive chest findings on auscultation, a high eosinophile count, and a documented or verified allergic history.

Immunology

UNDER THE DIRECTION OF MATTHEW WALZER, M.D., AND MURRAY ALBERT, M.D., BROOKLYN

The Correlation Between Anatomical Changes and the Allergic State in Tuberculous Guinea Pigs. Woodruff, E., and Kelly, R. G.: J. Immunol. **45**: 79, 1942.

The authors have previously shown that when tuberculous guinea pigs are kept completely desensitized by means of large daily doses of

tuberculin, the lungs show extensive pneumonic lesions containing large amounts of tubercle bacilli, and the liver and spleen are relatively normal.

In the present studies, guinea pigs were injected subcutaneously with virulent human tuberculin bacilli and were skin tested at frequent intervals to determine changes in skin sensitivity. The tissues of all animals which survived six weeks or longer were studied after death. It was found that the sensitivity rose gradually during the second week after infection, increased rapidly during the third week, and then leveled off. Those animals which had spontaneously lost their skin sensitivity to tuberculin before death showed extensive pulmonary lesions containing a large number of acid-fast bacilli but no caseation or necrosis of the alveolar walls. The spleens in the animals which had lost most of their allergy before death showed diffuse epithelioid cell infiltration and areas of infarction. Animals retaining a high degree of skin sensitivity to tuberculin before death showed few or no acid-fast bacilli in their pulmonary lesions. Some caseation was observed in the lungs of these animals, but these areas rarely contained tubercle bacilli.

It was concluded that in infected guinea pigs there is a distinct relationship between the degree of skin sensitivity to tuberculin before death and the pathologic findings in the lungs. Animals which are anergic before death show severe pulmonary lesions containing large numbers of acid-fast bacilli, while those which are allergic show few or no acid-fast bacilli in their pulmonary lesions.

Inhibitory Effect of Heparin Upon Histamine Release by Trypsin, Antigen, and Proteose. Dragstedt, C. A., and Rocha e Silva, M.: Proc. Soc. Exper. Biol. & Med. **51**: 101, 1942.

The symptoms observed in trypsin and peptone shock and in anaphylaxis are similar and are accompanied by a release of histamine into the plasma. The addition of antigen, peptone, or trypsin to heparinized rabbit's blood in vitro causes a release of histamine from the cells to the plasma. Heparin is known to be a trypsin antagonist. In an effort to determine whether the mechanism of the histamine release is the same with each of the three agents, heparin in excess of the amounts required to render the blood uncoagulable was employed as an inhibiting agent.

Bloods obtained from normal and sensitized rabbits were mixed with sufficient heparin to prevent clotting. To normal blood samples trypsin or proteose was added. To the blood from the sensitized animals, antigen (egg white) was added. In addition, heparin was added to each mixture. Adequate controls were employed. The mixtures were centrifuged, and the supernatant plasmas were assayed for histamine

content. The results indicated that the addition of heparin in amounts in excess of that required to render the blood incoagulable inhibited the release of histamine from the cells into the plasma in all samples, regardless of the cause of histamine release. Hence, it was concluded that the mechanism of histamine release was probably the same in trypsin and peptone shock and in anaphylaxis. It is suggested that heparin, when used in sufficient quantity, may inhibit anaphylactic shock in intact animals.

The Manufacture of Antibodies in Vitro. Pauling, L., and Campbell, D. H.: J. Exper. Med. **76**: 211, 1942.

Specific antibody was manufactured in vitro from normal serum globulin. Methyl blue was employed as the antigen and was mixed with the gamma globulin fraction of fresh beef serum. Sodium hydroxide was then added as a denaturing agent. After standing for from fifteen to thirty minutes, the mixture was dialyzed at 4° C. against a stream of phosphate buffer of pH 7.5. Thus the alkali-denatured protein molecules slowly assumed configurations, stable in neutral solution while in contact with antigen. The pH of the protein-antigen solution changed from 11.0 to 7.5, and small amounts of protein-dye precipitate were obtained which were found to be the result of antigen-antibody reaction. It was impossible, however, to separate the dye from the dye-protein precipitate to obtain a specific precipitin for the dye. Antibodies homologous to the azophenylarsonic group and to pneumococcus polysaccharide type III were also manufactured in vitro from various serum proteins by denaturation-renaturation processes. When pneumococcus polysaccharide type III was employed as the antigen, the resulting antisera precipitated type III polysaccharide and agglutinated type III pneumococci. Types I and VIII polysaccharide were not precipitated, and pneumococci types I and II were not agglutinated by the manufactured antiserum. It was concluded that normal serum globulin can be converted into antibody in vitro when it is denatured and renatured in the presence of antigen. Denaturation can be accomplished by heat or chemical action.

Pediatrics

UNDER THE DIRECTION OF WILLIAM C. DEAMER, M.D., SAN FRANCISCO

A Series of Substitutes for Milk in the Treatment of Allergies. Wolpe, **L. Z., and Silverstone, P. C.**: J. Pediat. **21**: 635, 1942.

Nine milk substitute formulas are offered and an analysis of their composition given. These formulas are constructed from oat, barley, soy, lima bean, pea, taro, rice, rye, or corn flours with the addition of cottonseed, olive, sesame, corn, peanut, or soy oil, and gelatin, dextrose,

imitation vanilla, salt, bone phosphate, or dicalcium phosphate, and of a 10 per cent solution ferric chloride and saccharin.

A vitamin powder was prepared for addition to the formula chosen. In addition to the use of a milk-free diet, instructions are given for the maintenance of a dust-free bedroom for the patient, and various local measures used, including compresses, Lassar's paste, and coal tar.

The authors evidently feel that milk allergy is so important in the etiology of infantile eczema that no other course is open but to eliminate all milk in these cases if a trial of evaporated or goat's milk fails. The dust directions are given not with regard to the eczema but to help avoid future allergies. A brief outline of twenty instances in most of which milk elimination apparently benefited infantile eczema is given. Skin tests are not mentioned. Only two of the twenty patients were hospitalized. Of the flours, oat and barley were most frequently tolerated, and of the oils, olive and sesame.

Some Comments on Asthma and Allergic Rhinitis in Childhood. Deamer, W. C.: Clinics **1**: 655, 1942.

This article is a general discussion intended for those who encounter clinical allergy in their practice but do not specialize in allergic diseases. The pitfalls of recognizing asthma and allergic rhinitis, especially in history taking, are mentioned. The frequent misinterpretation of allergic rhinitis as a common cold and the resultant assumption of a state of bacterial allergy if asthma follows is pointed out. The value of "continuity symptoms," such as cough, sneezing, and nasal itching in judging progress, are emphasized. Emphasized also is the desirability of meticulous care of the bedroom where almost half of the twenty-four hours of the day are spent, if an "environment trial" indicates that house dust is a factor.

Allergy in Childhood. V. Choice of Drugs in the Treatment of the Asthmatic Attack. Ratner, B.: New York State J. Med. **42**: 2029, 1942.

Adrenalin should never be given children in doses larger than 3 minims, and it should be given subcutaneously or intracutaneously, never intramuscularly or intravenously. There is no place for adrenalin in oil or 1 to 100 adrenalin inhalations, and both can be dangerous. Adrenalin may separate from oil and is especially habit forming when offered as an inhalant. It often causes severe adominal crises after inhalation. Syrup of ipecac by mouth in doses sufficient to cause vomiting is of value especially in asthma due to bronchial obstruction, and it should be used more extensively. It aids in increasing peristaltoid action in the bronchial tree and thereby releases plugs. Chest x-rays of the same child taken two hours apart have shown the disappearance of extensive atelectasis following the use of ipecac.

Most deaths resulting from asthma have been directly or indirectly due to the use of morphine, the use of which in asthma is little short of criminal. Histamine and histaminase as well as aminophyllin have little place in the treatment of asthma in childhood.

Cough in the Allergic Child. Marks, M. B.: Arch. Pediat. **59**: 697, 1942.

A child who coughs longer than six weeks and who does not have whooping cough should be investigated from the standpoint of allergy. While the examination of the chest is usually negative, nasal signs and symptoms of the allergy are often present. Much can be accomplished by simple measures such as the creation of a nonallergic environment and the use of an arbitrary trial diet. If skin testing is considered necessary, thirty or forty tests usually suffice. An environmental allergic program should be carried out even though negative tests are obtained. Patients sensitive to house dust should be tested with extract every five days, and its effectiveness is enhanced if a bacterial vaccine is also given. Infection of the nasopharynx and sinuses must be adequately treated and endocrine dysfunction corrected. If these measures fail, nonspecific ones may be tried including a change of climate.

Rhinology and Ophthalmology

UNDER THE DIRECTION OF FRENCH K. HANSEL, M.D., ST. LOUIS

Efficacy of Vasoconstrictor Agents in the Obstructed Nose. Sternstein, H. J.: Arch. Otolaryng. **36**: 713, 1942.

In studying the efficacy of vasoconstrictor agents in the obstructed nose, Sternstein concludes that the shrinkage efficiency of a drug is diminished in proportion to the pathologic degree of erectile tissue swelling. He found that ephedrine hydrochloride in aqueous solutions is more effective than cocaine hydrochloride, except when the latter is combined with ephedrine hydrochloride, in the treatment of acute infections of the upper respiratory tract, chronic hypertrophic rhinitis, and polypoid conditions with associated fixed tissue changes. He also noted that the synthetic derivatives of ephedrine and its analogues, such as amphetamine (benzedrine) and neosynephrin hydrochloride are more effective than the natural ephedrine. Of the two drugs, neosynephrin hydrochloride 0.25 per cent appears to be the more efficient vasoconstrictor agent, judged by its comparative action in the nonobstructed nose.

Present Status of Roentgen Therapy in Chronic Paranasal Sinusitis. Butler, Frank E., and Woolley, I. M.: Radiology **39**: 69, 1942.

In a review of published statistics on the results of roentgen therapy in chronic paranasal sinusitis, Butler and Woolley report that 33 per

cent of patients were cured and 41 per cent definitely improved by treatment. In their eleven years of experience with roentgen therapy of chronic sinusitis, they have never seen any harmful results. The best results were obtained in those cases in which roentgenograms show a marked thickening of the sinus membranes with a small central pneumatized cavity. No distinction was made between those cases of chronic sinusitis due to infection and those due to allergy.

Paralysis of Left Recurrent Laryngeal Nerve Following Subcutaneous Administration of Antitetanic Serum. Floyd, H. S., Pembleton, W. E., and Vinson, P. P.: West Virginia M. J. **38**: 253, 1942.

Floyd, Pembleton, and Vinson report a case of paralysis of the left recurrent laryngeal nerve following the subcutaneous injection of antitetanic serum. The patient was a man, 24 years of age, who had been given 1,500 units of antitetanus serum after a lacerating injury of the hand. The skin test for sensitivity to the serum had been negative, but eight days later serum sickness developed, complicated by paralysis of the brachial plexus and of the recurrent laryngeal nerve on the left side. Examination of the larynx showed a complete paralysis of the left vocal cord. The paralysis gradually cleared up as the general symptoms subsided. The larynx was normal six months after the onset.

Mucosography of the Respiratory Tract. Farinas, Pedro L.: Radiology **39**: 84, 1942.

Farinas describes a method for studying the condition of the mucosa of the respiratory tract by vaporization of an opaque medium (Merck's 40 per cent solution of neo-iodipin), employing an atomizer introduced behind the epiglottis with the aid of a laryngeal mirror. Radiograms are made immediately after the introduction of the opaque medium.

Only 5 c.c. of the solution are used for examination of the larynx and upper trachea. For the lower trachea and bronchi, about 15 c.c. should be employed. By this method the normal structures of the larynx may be demonstrated in both lateral and sagittal films. The outlines of the trachea and bronchi are clearly shown by thin dense lines, contrasting with the air in the lumen. A tumor in the trachea may be outlined by the opaque medium. If there is an infiltrating process in the larger bronchi, an alteration in the contour indicates its presence, even if the caliber of the bronchial lumen is not reduced. A lesion of this type is not demonstrated by the usual method of bronchography. This method of introducing the opaque medium is well tolerated by the patient.

Endophthalmitis Phacoanaphylactica and Its Relation to Sympathetic Ophthalmia. de Veer, J. A.: Arch. Ophth. **23**: 237, 1940.

De Veer reports his observations on a type of intraocular inflammatory reaction that occasionally follows accidental, surgical, or spontaneous rupture of the crystalline lens. He noted that clinical reactions to lens substance occurred among 8 per cent of the patients who showed positive reactions to intracutaneous injections of lens antigen. He suggests that routine tests be performed on all subjects who are to undergo cataract operations or on those with a previous history of lens injury. In these cases, desensitization with the antigen, augmented by staphylococcus toxin, is indicated. It is suggested that prevention of phacoanaphylactic reactions through desensitization will result in reduction of the incidence of sympathetic ophthalmia, especially of that incident to cataract extraction.